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THE RELATIVE EFFECTS OF DESOXYCORTICOSTERONE AND WHOLE CORTICO-ADRENAL EXTRACT ON ADRENAL INSUFFICIENCY[1]

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Observations made in the past three years on the relative effects of desoxycorticosterone and cortico-adrenal extract on blood-chemical and other conditions in adrenal insufficiency have been somewhat confusing. With increasing utilization of these materials in clinical conditions, it is highly desirable that a more exact knowledge of their influence be secured. A year ago we reported some preliminary observations on this subject (Britton and Corey, 1940), and these have now been extended to include further experimental series and more rigorous test methods. Desoxycorticosterone acetate[2] has been compared physiologically with whole cortico-adrenal extract of high purity prepared in this laboratory (Britton and Silvette, 1931).

METHODS. In all cases except normal controls, tests were made on animals from which both adrenal glands had been removed. Small amounts of cortico-adrenal extract, and also sodium chloride in the drinking water, were first given post-operatively to all animals for about a week. Treatment was then stopped, and usually within two or three days, when early symptoms of adrenal insufficiency appeared, studies were made of hormonal effects (see details later). Cats and rats were used.

Blood glucose was determined by the method of Folin and Malmros (1929) and glycogen by a modified Pflüger technique (Silvette and Britton, 1932). Analyses for sodium were made by the method of Butler and Tuthill (1931); for potassium, Kramer and Gittelman (1926); for chloride, Van Slyke and Sendroy (1923); and for urea, Van Slyke and Kugel (1933).

[1] Grateful acknowledgment is made of aid received in this investigation from the Committee on Research in Endocrinology of the National Research Council.

[2] Desoxycorticosterone acetate was very kindly and generously supplied under the name "Cortate" by the Schering Corporation.

RESULTS. *Cats.* Effects on the general condition of animals after injection are given in table 1. It was evident throughout all the experimental series on cats that while desoxycorticosterone usually abolished the early symptoms of adrenal insufficiency and restored tissue and blood-

TABLE 1

General effects produced by (A) desoxycorticosterone and (B) cortico-adrenal extract on adrenalectomized cats with symptoms of insufficiency

CAT NO.	AMOUNT MATERIAL INJECTED	INITIAL CONDITION OF CAT	RESULTS
		(A) Desoxycorticosterone	
	mgm.		
1	25	Weak	Improved, but would not eat in 6 hours
2	25	Sluggish	Slight improvement 6 hours after injection
3	25	Sluggish	Animal interested in food in 7 hours
4	25	Sluggish	Improved but would not eat in 7 hours
5	25	Weak	Appeared fairly normal within 8 hours
6	25	Weak	Slight improvement at end of 6 hours
7	25	Weak	Slightly improved after 6 hours
8	50	Weak	Improved at end of 8 hours, normal at 16 hours
9	50	Weak	Normal but would not eat at 16 hours
10	50	Weak	Normally active but not eating at 16 hours
11	20	Sluggish	Slight improvement at end of 6 hours
12	20	Sluggish	Slight improvement at 8 hours
13	20	Sluggish	Condition unchanged at 8 hours
14	20	Sluggish	Somewhat improved at end of 8 hours
15	20	Sluggish	Improved after 6 hours, would not eat at 8 hours
		(B) Cortico-adrenal extract	
	cc.		
21	50	Weak	Animal eating at end of 6 hours; normal
22	50	Weak	Improved, interested in food at end of 2 hours
23	25	Convulsions	Took food at end of 2 hours
24	25	Comatose	Normally active at 1 hour, eating at 2 hours
25	25	Convulsions	Normal, ate meal of salmon at 3 hours
26	25	Convulsions	Appeared normal at 1 hour, ate at 2 hours
27	25*	Convulsions	Improved at 1 hour, eating at 2 hours
28	20*	Weak	Took meal at end of 4 hours; normal
29	20*	Convulsions	Normal, eating, at end of 1 hour
30	10*	Convulsions	Normal, took food at end of 1 hour

* Extract given orally.

chemical conditions within 24 hours, whole cortico-adrenal extract was a much more rapid and efficacious agent. Large doses of desoxycorticosterone brought about no general improvement in cats with symptoms of adrenal insufficiency in less than 6 hours, in 15 experiments. In only one

case was an animal restored sufficiently to accept food (canned salmon) within 16 hours after injection. In a few cases with severe symptoms, desoxycorticosterone was unable to bring about restoration. The material was also not active in two cases treated by mouth. Whole cortico-adrenal extract appeared markedly effective, in contrast, even in cases of extreme insufficiency (10 experiments). Sometimes within an hour after administration, extract-treated animals appeared normal and ate well. Oral treatment (extract) was equally effective (see Britton, Flippin and Silvette, 1931).

Comparison was made of changes in serum electrolytes and blood and tissue glycogen brought about by desoxycorticosterone and cortico-adrenal extract respectively, in cats with adrenal insufficiency. Slight weakness only was allowed to develop before treatment. Blood samplings were made before injection (0 hour), and at the end of 8 and 16 hours in different series. Twenty-five milligrams of desoxycorticosterone or 25 cc. of cortico-adrenal extract per 8 hours were administered. Changes in serum electrolytes (K, Na, Cl) toward normal levels were observed in all cases at the end of 8 hours. Blood sugar and liver glycogen levels were still subnormal, however, at the end of 16 hours after desoxycorticosterone administration (7 cases). In the same period, the carbohydrates were approximately normal after cortico-adrenal extract treatment (4 cases). The respective readings were: 16 hours after desoxycorticosterone: blood sugar, 74 mgm. per cent; liver glycogen, 0.22 per cent; 16 hours after cortico-adrenal extract: blood sugar, 98 mgm per cent; liver glycogen, 0.89 per cent. In all cases the muscle glycogen values were within normal limits.

Adrenalectomized cats which were allowed to develop signs of insufficiency, and then treated with small amounts of desoxycorticosterone (5 mgm.) twice daily for $3\frac{1}{2}$ days, showed normal electrolyte and carbohydrate levels. Extract-treated animals observed under similar conditions displayed higher carbohydrate readings. The results were as follows: At end of $3\frac{1}{2}$ days' treatment with desoxycorticosterone (ave. 3 cases): blood sugar, 86 mgm. per cent; liver glycogen, 0.83 per cent. After $3\frac{1}{2}$ days' treatment with cortico-adrenal extract (ave. 5 cases): blood sugar, 102 mgm. per cent; liver glycogen, 1.06 per cent.

In further tests desoxycorticosterone was given in fairly large dosage with glucose-saline to adrenalectomized cats with slight insufficiency symptoms and the effects compared with those produced by whole extract. Considerable difference will be observed in the results in these two series (table 2). In the 8-hour experimental period under the influence of cortico-adrenal extract, large deposits of glycogen were formed in the liver, heart and skeletal muscle, and serum electrolyte and blood-urea levels were restored to approximately normal. In the case of desoxycorticosterone administration, however, even after injecting 20 mgm., the carbohydrate

(blood sugar excepted) and electrolyte and urea levels were not far removed from those found in adrenal insufficiency.

In table 3 several pertinent averages are compared. Marked differences in the extent of influence of desoxycorticosterone and adrenal extract are apparent. The activity of the former single adrenal factor is relatively very slight in comparison with that of the whole cortico-adrenal complex.

Rats. Two groups of adrenalectomized rats were also given desoxycorticosterone and glucose, and the results compared with a series treated with extract and glucose (table 4). Again, the electrolyte and carbohydrate

TABLE 2

Effects of desoxycorticosterone and whole cortico-adrenal extract on carbohydrate and electrolyte levels in adrenalectomized cats with early insufficiency symptoms

All animals given 1 per cent body weight of 3 per cent glucose in 0.9 per cent NaCl, (A) plus desoxycorticosterone (5 mgm.) and (B) plus cortico-adrenal extract (10 cc., made up to volume with the glucose solution), every 2 hours for an 8-hour period. Samples taken at end of 8 hours.

CAT NO.	BLOOD SUGAR	GLYCOGEN			SERUM ELECTROLYTES			BLOOD UREA
		Liver	Muscle	Heart	K	Na	Cl	
(A) Desoxycorticosterone								
	mgm. per cent	*per cent*	*per cent*	*per cent*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*mgm. per cent*
40	97	0.29	0.30	0.32	7.17	139.9	112.4	53
41	98	0.26	0.28	0.34	7.80	138.9	110.6	70
42	110	0.19	0.30	0.37	8.11	137.1	113.8	72
43	96	0.30	0.35	0.35	7.01	138.3	111.8	58
44	100	0.22	0.33	0.41	8.13	136.9	109.6	60
(B) Cortico-adrenal extract								
45	142	1.98	0.58	0.72	6.11	149.9	119.8	40
46	191	2.01	0.49	0.62	7.01	150.1	118.8	46
47	160	1.24	0.53	0.67	6.93	148.3	120.3	49
48	134	1.60	0.58	0.63	5.19	152.3	120.6	52
49	146	1.88	0.49	0.73	6.37	149.5	118.8	44
50	140	2.00	0.57	0.61	5.77	149.7	119.6	51

levels in the extract-treated animals were found to be practically normal at the end of an 8-hour experiment. Readings in the desoxycorticosterone group were in contrast not greatly different from those observed in the glucose-saline treated controls.

Discussion. From most of the recent evidence it appears that desoxycorticosterone is able to maintain electrolyte balance in cases of adrenal insufficiency, both clinical and experimental. It is questionable, however, whether it influences notably the carbohydrate levels. Animals without adrenal glands may be kept alive at least for long periods on relatively

small doses of the substance, although it has been recommended that glucose be added liberally to the diet.

Shortly after Reichstein prepared the crystalline material its biological potency was shown on dogs and rats; it was recognized as considerably inferior, however, to corticosterone (Steiger and Reichstein, 1937). In several experimental and clinical studies, Thorn and his colleagues (1938–40) have emphasized the important influence of desoxycorticosterone.

Harrison and Harrison (1939) have found that considerable amounts of desoxycorticosterone are able to keep up the blood sugar in adrenalectomized rats. Further, FitzGerald and Verzar (1939) stated that under some conditions the substance may keep up liver glycogen in hypophysectomized animals. Hartman and his colleagues (1940) noted, however,

TABLE 3

Average carbohydrate and electrolyte levels in cats under different conditions

CONDITIONS	NO. OF CATS	BLOOD SUGAR	GLYCOGEN			SERUM ELECTROLYTES			BLOOD UREA
			Liver	Muscle	Heart	K	Na	Cl	
		mgm. per cent	*per cent*	*per cent*	*per cent*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*mgm. per cent*
Normal, fasting 24 hours	10	88	1.22	0.43	0.61	5.20	155.6	123.4	40
Adrenalectomized, showing symptoms, untreated	10	57	0.07	0.21	0.21	9.30	133.0	109.2	85
Adrenalectomized, desoxycorticosterone treated (table 2)	5	100	0.25	0.31	0.36	7.66	138.2	111.6	63
Adrenalectomized, cortico-adrenal extract treated (table 2)	6	152	1.77	0.54	0.66	6.23	150.0	119.6	47

that while "cortin" gave protection against insulin convulsions in mice, desoxycorticosterone was ineffective. In agreement are the observations of Jensen and Grattan (1940).

Anderson and Herring (1940) observed that saline solution maintained glycogen stores in the adrenalectomized rat equally as well as desoxycorticosterone. Recently we have shown (Corey and Britton, 1941) that while whole cortico-adrenal extract produces marked glycogenesis in the isolated cat liver, desoxycorticosterone is quite ineffective.

It is apparent from the present extended series of experiments that desoxycorticosterone occupies a considerably inferior position to whole cortico-adrenal extract in the treatment of experimental adrenal insufficiency. Although some good effects of desoxycorticosterone on the

TABLE 4

The influence of desoxycorticosterone and cortico-adrenal extract on carbohydrate and electrolyte levels in adrenalectomized rats

Animals maintained on glucose-saline solution for 1 week after operation, and treatment given 2 days later. Injections given in all cases at 0, 2, 4 and 6 hours, on the basis of 100 grams body weight. Tissues used at 8 hours.

CONDITIONS	RAT NO.	BLOOD SUGAR	GLYCOGEN			SERUM K	SERUM Na
			Liver	Muscle	Heart		
Desoxycorticosterone treated							
		mgm. per cent	*per cent*	*per cent*	*per cent*	*m.e./l.*	*m.e./l.*
5 mgm. plus 5 cc. 3 per cent glucose in 0.9 per cent NaCl per injection.	1	190	0.53	0.35	0.29	7.99	141.1
	2	194	0.52	0.39	0.20	7.99	139.9
Averages		192	0.53	0.37	0.25	7.99	140.5
5 mgm. plus 2 cc. 3 per cent glucose in 0.9 per cent NaCl per injection.	3	97	0.42	0.37	0.29	7.17	142.5
	4	100	0.57	0.42	0.30	7.77	140.3
	5	99	0.48	0.44	0.32	7.91	140.7
	6	106	0.42	0.38	0.42	8.29	144.3
	7	104	0.49	0.41	0.28	7.49	138.5
Averages		101	0.48	0.40	0.32	7.73	141.3
Cortico-adrenal extract treated							
5 cc., made up with 3 per cent glucose and 0.9 per cent NaCl per injection	8	426	1.31	0.61	0.26	6.89	147.3
	9	385	1.29	0.51	0.41	6.17	149.1
	10	500	1.57	0.56	0.27	6.49	148.3
Averages		437	1.39	0.56	0.31	6.52	148.2
2 cc. with glucose-saline as above	11	144	0.82	0.61	0.36	6.01	149.9
	12	120	1.01	0.49	0.41	6.11	148.1
	13	112	0.83	0.53	0.54	7.01	150.1
	14	112	0.91	0.56	0.33	6.53	147.7
	15	109	0.76	0.56	0.44	5.79	149.9
Averages		119	0.87	0.55	0.42	6.29	149.1
Control animals (adrenalectomized)							
5 cc. glucose-saline solution as above, without hormone (average 2 cases)		146	0.26	0.44	0.25	8.08	135.8
2 cc. glucose-saline (average 3 cases)		96	0.15	0.30	0.25	8.05	134.6
Normal rats, non-fasting, untreated (average 10 cases)		108	1.41	0.62	0.70	6.32	141.2

early symptoms are observed, they are usually slowly produced over many hours; and in severe cases, restoration does not occur.

It appears hardly likely that any direct or specific influence on carbohydrate metabolism is brought about through desoxycorticosterone action, the slow changes that are observed probably being explicable on the basis of favorable correlated electrolyte shifts. No effects on the high blood-urea levels are produced by the material, in contrast to the sharp reductions which follow extract injection. Desoxycorticosterone appears to exert its action chiefly and perhaps only on salt and water balance in the body.

SUMMARY

Desoxycorticosterone given to animals (cats) showing early symptoms of adrenal insufficiency acts slowly over a period of 6 to 24 hours. It usually but not always effects recovery to normal. Animals with severe insufficiency do not respond to desoxycorticosterone treatment alone. Oral treatment is not effective. In contrast, whole cortico-adrenal extract restores animals with severe adrenal insufficiency (convulsions, coma) in 1 to 6 hours, and oral and other routes of administration are effective.

In adrenalectomized cats with slight insufficiency, desoxycorticosterone restored serum electrolyte levels to approximately normal in 16 hours. There were still deficiencies in carbohydrate levels, however, at this time. Under the same conditions, cortico-adrenal extract brought about restitution of normal carbohydrate and electrolyte values.

Over a period of $3\frac{1}{2}$ days, desoxycorticosterone was able to restore and maintain normal carbohydrate and electrolyte levels in adrenalectomized cats. Blood sugar and liver glycogen values in extract-treated cases were, however, much higher.

Adrenalectomized cats with symptoms of insufficiency continued to show disturbed glycogen, electrolyte and urea levels after treatment for 8 hours with desoxycorticosterone and glucose-saline solution. Blood sugar levels alone were normal. Adrenal extract under the same conditions brought about complete restitution of normal values.

Experiments similar to the above made on adrenalectomized rats also demonstrated the inability of desoxycorticosterone used with glucose-saline to restore normal electrolyte and carbohydrate levels, in an 8-hour period. This was in sharp contrast to the restorative action of cortico-adrenal extract.

The single crystalline adrenal factor, desoxycorticosterone, given routinely in moderate dosage, maintains normal tissue and blood-chemical values in adrenalectomized animals. Its action is much inferior to the whole cortico-adrenal hormonal complex, however, in restoring animals in the crisis of insufficiency. Desoxycorticosterone probably has no direct influence on carbohydrate metabolism.

In the crisis of adrenal insufficiency, if whole cortico-adrenal extract is not utilized, the advisability of using glucose in conjunction with desoxycorticosterone is strongly indicated.

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THE ANTAGONISTIC ACTION OF DESOXYCORTICOSTERONE AND POST-PITUITARY EXTRACT ON CHLORIDE AND WATER BALANCE[1]

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Important relationships between the adrenal cortex and the post-pituitary gland in their influence on body water and electrolytes have been shown in several papers published from this laboratory in the past few years (Silvette, 1937, 1938; Silvette and Britton, 1938; Corey, Silvette and Britton, 1939). That there is a specific hormone of the adrenal cortex which acts on the kidney to produce diuresis, antagonizing the influence of the post-pituitary antidiuretic factor, has also been indicated (Silvette and Britton, 1938). In the present experiments we have considered further the hypophyseal factor and more particularly the action of desoxycorticosterone on the rat, extending our earlier work with whole cortico-adrenal extract used on the opossum. Experiments were carried out on normal, hypophysectomized and adrenalectomized rats, using desoxycorticosterone, post-pituitary extracts and other substances in various series.

METHODS. Fluid exchanges were determined by the use of individual metabolism cages fitted with graduated drinking tubes; urine was collected under toluene in graduated cylinders, and the urinary chloride concentrations determined by means of the Volhard titration. Metabolism tests were run for 12-hour periods in all instances with water (or other drinking solutions as indicated later) available at all times. Two to four days were allowed for "rest" between runs. Fasting periods were not observed before the metabolism tests, except in a few groups, in which no significant differences were found. Desoxycorticosterone and post-pituitary extract were injected subcutaneously, the former in 2 mgm. doses every 2 hours, and the latter $\frac{1}{2}$ u. initially and $\frac{1}{4}$ u. subsequently at similar intervals.

RESULTS. *Normal Rats.* More than 100 normal male rats were utilized in various experiments, preliminary to tests of hypophysectomized and

[1] Grateful acknowledgment is made of aid received in this investigation from the Committee on Research in Endocrinology of the National Research Council.

adrenalectomized animals. The results of these experiments are summarized below (table 1 A).

Desoxycorticosterone injections in the rat allowed only water to drink resulted in a moderate "diabetes insipidus" with chloride retention in all animals tested. When desoxycorticosterone was administered together with saline solution the effect on fluid exchange was more definite, although hyperchloruria attributable to NaCl intake was now apparent.

The effects of desoxycorticosterone and post-pituitary extract on urinary sodium excretion were tested under similar metabolic conditions in a few groups of cases. All the rats were unoperated normals, and the following were the results:

Desoxycorticosterone injected: 11 cases; av. urine sodium 0.35 mgm. per cc.

Post-pituitary extract injected: 7 cases; av. urine sodium 4.16 mgm. per cc.

Untreated controls: 6 cases; av. urine sodium 1.19 mgm. per cc.

In some cases in which pitressin was used, the results were somewhat similar to those produced by whole post-pituitary extract.

Hematocrit determinations in those cases in which desoxycorticosterone was injected were in agreement with the effects on water balance. In 7 animals, the total blood cell volumes fell continuously, although slightly, over the 12-hour metabolic period. Following post-pituitary extract injection, there were no significant changes observed in the hematocrit readings (8 cases).

Post-pituitary extract administration to normal rats resulted in an antidiuresis with extreme hyperchloruria—an effect directly antagonistic to that produced by desoxycorticosterone. The presence of NaCl in the drinking solution opposed or masked the antidiuretic effect of the post-pituitary principle, and the resultant hyperchloruria was somewhat decreased although still severe. It was clear that desoxycorticosterone and post-pituitary extract, when injected into normal male rats, produced opposite effects as regards water and electrolyte balance.

When saline or glucose solutions were used in the drinking tubes the changes in fluid exchange were approximately equal in extent, with pronounced hyperchloruria in the former case, however, and chloride retention in the latter. The combination of the two substances in the drinking solutions produced marked increases in water intake and urine output, and severe hyperchloruria. It should be noted that glucose-solution feeding produced results essentially similar to those which followed desoxycorticosterone treatment.

Hypophysectomized Rats. Course of diabetes insipidus. In earlier studies (Corey, Silvette and Britton, 1939) we were particularly concerned with the phase of acute diabetes insipidus which immediately follows pituitary ablation and persists for a few days afterwards. We have

TABLE 1

Effects of desoxycorticosterone and post-pituitary extract on fluid balance in the rat under various conditions

NO. OF CASES	TREATMENT	AVERAGE WATER INTAKE	AVERAGE URINE OUTPUT	AVERAGE URINARY CHLORIDE
	A. Normal rats			
		cc./100 grams weight	*cc./100 grams weight*	*mgm./cc.*
37	Water ad lib.	1.7	1.4	2.80
15	0.9 per cent NaCl ad lib.	4.5	2.4	6.44
8	2.0 per cent glucose ad lib.	4.3	2.7	1.41
8	0.9 per cent NaCl—2.0 per cent glucose ad lib.	10.7	7.4	7.39
12	Desoxycorticosterone; water ad lib.	2.9	1.9	1.50
10	Desoxycorticosterone; 0.9 per cent NaCl ad lib.	7.5	4.6	7.34
10	Post-pituitary extract;† water ad lib.	0.6	1.1	16.20
8	Post-pituitary extract; 0.9 per cent NaCl ad lib.	4.3	3.3	9.19
6	Post-pituitary extract; 2.0 per cent glucose plus 0.9 per cent NaCl ad lib.	5.0	4.4	9.76
	B. Hypophysectomized rats			
26	Water ad lib.	2.3	1.9	2.12
9	0.9 per cent NaCl ad lib.	14.1	7.9	3.58
12	2.0 per cent glucose ad lib.	11.0	7.5	0.51
6	0.9 per cent NaCl—2.0 per cent glucose ad lib.	12.4	8.0	1.47
52	Desoxycorticosterone; water ad lib.	5.1	5.2	0.38
6	Desoxycorticosterone; 0.9 per cent NaCl ad lib.	10.2	5.4	5.38
10	Post-pituitary extract; water ad lib.	0.8	1.7	6.40
8	Post-pituitary extract; 0.9 per cent NaCl ad lib.	2.0	2.3	6.63
12	Desoxycorticosterone—post-pituitary extract; water ad lib.	1.9	1.9	6.09
	C. Adrenalectomized rats			
10	Water ad lib.	3.4	2.1	3.30
12	0.9 per cent NaCl ad lib.	6.8	2.5	9.17
14	2.0 per cent glucose ad lib.	10.7	6.3	1.25
24	0.9 per cent NaCl—2.0 per cent glucose ad lib.	15.0	7.4	7.96
7	0.9 per cent NaCl—5.0 per cent glucose ad lib.	22.1	15.0	7.19
11	Desoxycorticosterone; water ad lib.	7.8	5.0	1.11
13	Desoxycorticosterone; 0.9 per cent NaCl ad lib.	16.5	7.1	5.04
6	Post-pituitary extract; water ad lib.	0.9	1.3	8.25
11	Post-pituitary extract; 0.9 per cent NaCl ad lib.	7.9	3.6	10.32

* Desoxycorticosterone acetate ("Cortate"), generously supplied by the Schering Corporation; injected subcutaneously, 2 mgm. every 2 hours.

† Post-pituitary extract (posterior pituitary solution Squibb), generously supplied by E. R. Squibb and Sons; injected subcutaneously, ½ u. initially and ¼ u. every 2 hours subsequently.

In some cases tested, considerably smaller doses given less frequently yielded similar results.

emphasized in other reports the transitory nature of this diabetic condition in the rat. As a preliminary to experiments on "chronic" hypophysectomized animals, however, we extended our studies to include more protracted periods up to 80 days after operation. The marked diabetic state immediately following operation in the rat is admittedly a transient and unstable one, and investigation of the "chronic" condition was considered highly desirable.

A study of the averages in our data led to a division of the post-operative diabetic state seen in the hypophysectomized rat into three stages as indicated in table 2: *1*, a primary acute condition persisting for 3 to 5 days, in which polyuria predominated and the water-intake/urine-output ratio (W/U) was less than unity; *2*, a secondary (but still acute) phase of about 3 days' duration in which an emerging polydipsia became evident and during which water intake exceeded urine output, with an average W/U ratio for the period of more than one; and *3*, a tertiary "chronic" condition of mild diabetes in which the hypophysectomized animals showed a slight but constant increased water intake and urine output, with a W/U ratio almost identical with that of normal controls.

It was apparent from this extended series of cases that experimental diabetes insipidus in the rat is characterized by an immediate and marked primary polyuria; then, usually within 12 to 24 hours after operation, polydipsia is observed. These conditions are acute in character, and accompanied by reduced urinary chloride concentration. Subsequently there appears a change to a type of water balance in which the polydipsia predominates. This moderately diabetic condition may persist until death of the animal, or at least until an extreme state of inanition is reached. The chronic state shows fluid and chloride levels approaching the normal, but careful inspection of the values always reveals the persistence of at least a slight diabetic condition.

Desoxycorticosterone and post-pituitary extract effects on hypophysectomized rats. Tests of the effects of injections of desoxycorticosterone were made repeatedly on a series of 12 hypophysectomized rats, over a period of several weeks after operation. The influences on fluid exchange are presented graphically in figure 1. It may be noted that in the untreated cases at the end of the first post-operative week, water intake and urine output had reached levels only slightly above the normal. Injection of desoxycorticosterone on the 12th and 26th post-operative days resulted in dramatic augmentation of the polydipsic and polyuric condition to levels approximating those seen immediately after operation. In repeated tests on the 32nd and 39th days, definite but somewhat smaller reactions were observed. On the 62nd day the diabetic response was again evoked by means of desoxycorticosterone, the results differing only slightly from those observed on the 39th day. The urinary chlorides in these cases were greatly reduced

by desoxycorticosterone, in reciprocal correlation with the marked increases in fluid exchange.

While it is evident therefore that desoxycorticosterone injection over a period of two months or more after hypophysectomy resulted invariably in a return toward the primary acute state seen during the first few days after operation, it was noted that the responses to the injections became successively less in repeated experiments.

In all, 52 hypophysectomized rats were treated with desoxycorticosterone at intervals from 12 to 80 days after operation (table 1 B). The averages for all cases, when compared with 26 untreated hypophysectomized animals, revealed that the injections produced a marked condition of diabetes insipidus and an attendant hypochloruria. Thus, it was found that desoxycorticosterone given to hypophysectomized rats augmented

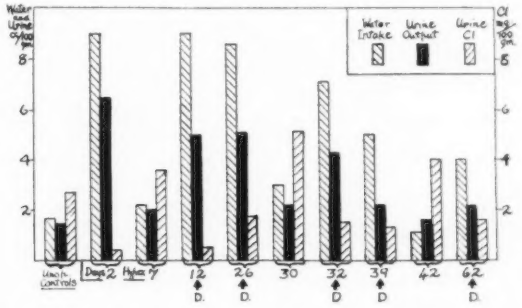

Fig. 1. Influence of desoxycorticosterone on water intake, urine output and urinary chlorides in hypophysectomized rats at different time periods after operation. Injections were given on days marked with arrows (D). Comparison is made with unoperated controls.

fluid intake by over 100 per cent and urine output by nearly 200 per cent, while it also produced a specific retention of chlorides. Compared with normal untreated rats, the polydipsia and polyuria were calculated as approximately 200 and 250 per cent respectively. It should be observed that no aggravation of the acute diabetic condition which immediately follows hypophysectomy could be induced by desoxycorticosterone administration.

Further examination of table 1 B shows that the effects brought about through post-pituitary extract injection were directly opposed to those produced by desoxycorticosterone. Thus, pituitary extract brought about a marked reduction in water ingestion, a decrease in urine output and a striking increase in chloride concentration (10 cases). When post-pituitary extract and desoxycorticosterone were administered simultaneously to the same animals, it was found that the former substance overwhelmed the

action of the latter, in the dosages employed. Hence the end result was a slight reduction in fluid exchange, and an increase in urinary chloride content almost as great as that seen in rats injected with post-pituitary extract alone.

Adrenalectomy. The water balance and urinary chloride excretion of 42 adrenalectomized rats were studied under different conditions from 12 hours to 18 days after operation. Examination of table 1 C shows that the urinary chlorides were increased post-operatively over untreated normal controls, although water intake and urine output were not greatly altered.

Desoxycorticosterone produced in adrenalectomized rats marked increases in fluid exchange (water intake and urine secretion), accompanied by severe restriction of chloride output. The conditions observed were similar to those which followed desoxycorticosterone administration to normal and also to hypophysectomized animals described above. The

TABLE 2

Course of diabetes insipidus and hypochloruria following hypophysectomy in the rat

PHASE OF DIABETES INSIPIDUS	NUMBER OF CASES	POST-OPERATIVE PERIOD	AVERAGE WATER INTAKE	AVERAGE URINE OUTPUT	AVERAGE URINARY CHLORIDE
		days	*cc./100 grams weight*	*cc./100 grams weight*	*mgm./cc.*
Primary acute	30	0– 5	6.3	7.1	0.34
Secondary acute	21	5– 8	3.6	2.9	1.14
Tertiary chronic	26	8–80	2.3	1.9	2.12
Normal controls	37		1.7	1.4	2.80

usual actions of saline and glucose solutions were observed, as in normal animals.

It may be noted that saline solutions did not augment water intake and urine output as greatly as did desoxycorticosterone, in these cases. The chloride-restricting action of the hormone also was not observed when NaCl was allowed in the drinking water. In adrenalectomized rats as well as all other cases, one should emphasize, glucose solutions brought about results similar to those produced by desoxycorticosterone, i.e., increased fluid intake and urine output and reduced urinary chlorides.

Post-pituitary extract effected exchanges in adrenalectomized rats similar to those in all other cases tested—water ingested and urine excreted were greatly reduced, concomitantly with increases in chloride concentration. Addition of NaCl to the drinking water, however, resulted in reversal of the fluid exchanges. Opposite actions of the post-pituitary principle and desoxycorticosterone were apparent in adrenalectomized animals as in all other conditions studied.

The above and other facts are shown comprehensively in table 3 here-

with. Quotations of changes on a percentage basis, and also in total amount of chloride excreted, give a striking picture of the counteracting effects of desoxycorticosterone and post-pituitary extract under the different conditions observed.

Discussion. In earlier reports (Silvette and Britton, 1938) the proposition has been put forward that in the excretion of water and salt by the kidney a diuretic hormone of the adrenal cortex acts in physiological antagonism to the antidiuretic hormone of the post-pituitary lobe. On a proper balance between the secretory activities of the adrenal cortex

TABLE 3

Differences in fluid balance and chloride output produced by desoxycorticosterone and post-pituitary extract

All animals allowed water ad lib. Calculations made from levels found in untreated animals in each of three groups.

NO. OF CASES	EXPERIMENTAL CONDITIONS	WATER INTAKE	URINE OUTPUT	URINARY CHLORIDE	URINARY CHLORIDES
		diff. per cent	*diff. per cent*	*diff. per cent*	*mgm./100 gram rat*
	Unoperated:				
34	Untreated (basals)				4.13
12	Desoxycorticosterone	+71	+27	−46	2.85
10	Post-pituitary extract	−65	−27	+148	17.82
	Hypophysectomized:				
26	Untreated (basals)				4.03
52	Desoxycorticosterone	+122	+174	−80	1.98
10	Post-pituitary extract	−65	−11	+204	10.88
	Adrenalectomized:				
10	Untreated (basals)				6.97
11	Desoxycorticosterone	+129	+138	−67	5.55
6	Post-pituitary extract	−74	−38	+150	10.72

and the post-pituitary gland, it is considered, fluid and electrolyte balance intimately depend. Further recent evidence supporting our work and proposals has been reviewed by Leiter (1941).

Much has been said of various disease complexes being due merely to the absence of one organ or another. For many decades diabetes mellitus was explained almost wholly on the basis of pancreatic disturbance and insulin lack; but the involvement of both pituitary and adrenal glands has now been generally acknowledged. "Hypophysists" and "hypothalamists" have vigorously discussed for some years the causative agents in diabetes insipidus, and no particular thought has been given to other possible etiological factors. It must nevertheless be admitted as a possibility that in

the case of removal or deficiency of either cortico-adrenal or post-pituitary tissues, the resultant fluid and salt disturbances may be explicable on the basis of the (one or other) unleashed or hyperactive and antagonistically-acting gland that may remain.

Cortico-adrenal extract and desoxycorticosterone have now been observed to produce a condition much like that of diabetes insipidus. Possibly, post-pituitary extract given in excess may be found to create a condition similar to that of adrenal insufficiency. The great loss of salt and restriction of water exchange produced by post-pituitary preparations are at least in agreement with this idea.

Besides the production of conditions similar to diabetes insipidus by cortico-adrenal principles, it has been shown (Corey, Silvette and Britton, 1939) that the d.i. state does not supervene after hypophysectomy if the adrenals are also removed at the same time. With the usual occurrence of diabetes insipidus on simple removal of the pituitary, there is a suggestion of cortico-adrenal influence—the course of water-balance disturbance goes hand-in-hand with hyperexcitation (in the first place) and later degeneration of the adrenal tissues.

With the above and also our earlier work, the reports of Martin and his associates (1939) and Ragan et al. (1940) are in essential agreement. Schweizer et al. (1940) have, however, noted some exceptions.

SUMMARY

Under the influence of desoxycorticosterone rats voluntarily drink more water and urine output is enhanced, while urinary chlorides are much reduced in concentration and total amount excreted. The reverse is true after post-pituitary extract injection—fluid exchanges being greatly reduced and chloride elimination markedly augmented. These conditions are seen alike in normal, hypophysectomized and adrenalectomized animals, observed over a 12-hour metabolism period.

Desoxycorticosterone was also found to reduce severely the output of urine sodium, while post-pituitary extract greatly increased its excretion.

When post-pituitary extract and desoxycorticosterone were administered together, the action of the former substance tended to overwhelm that of the latter.

Hemoconcentration followed desoxycorticosterone injection.

Glucose given in the drinking water brought about fluid exchanges and urinary chloride concentrations similar to those produced by desoxycorticosterone. Saline solutions given alone produced more marked increases in fluid intake and urine output than did desoxycorticosterone; when saline was given with desoxycorticosterone, the salt-restricting action of the latter was overcome. Also, when saline solutions were given to drink, post-pituitary extract did not reduce the fluid exchanges.

Studies were made chiefly in the chronic condition after either adrenalectomy or hypophysectomy. It is shown that the hypophysectomized rat (77 cases) passes through three post-operative phases: a primary acute condition lasting a few days in which polyuria predominates; a secondary but still acute phase in which polydipsia emerges; and a tertiary chronic state of mild diabetes insipidus with fluid exchanges always slightly above those in normal rats and urinary chlorides concomitantly subnormal. Possibly, altered activity of the cortico-adrenal tissues after hypophysectomy, or unchecked action of desoxycorticosterone, may account for the diabetes insipidus condition.

In a series of chronic hypophysectomized rats tested over a period of about 80 days, the action of desoxycorticosterone was found to become progressively less with repeated injections, possibly due to tolerance or anti-hormone effect.

It is apparent that the post-pituitary and cortico-adrenal tissues elaborate principles which specifically counteract or antagonize each other in their effects on fluid and electrolyte balance. For normal salt and water regulation in the body, a balanced relationship between the adrenal and pituitary mechanisms is therefore essential.

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THE INFLUENCE OF GELATIN INGESTION UPON THE CREATININE-CREATINE EXCRETION OF NORMAL MEN

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It is generally taught that creatine is not present in the urine of adult men apart from such pathological conditions as complete starvation, Graves' disease, and progressive muscle dystrophy. Attempts by various workers to bring about a creatinuria by feeding high protein diets have given conflicting results (Denis and Minot, 1917; Rose et al., 1918; Lewis and Doisy, 1918; Bollman, 1929–30). Following the demonstration by Brand et al. (1929) that feeding glycine to persons with progressive muscle dystrophy increased their output of creatine, several investigators fed glycine to normal individuals (rats and man) (Bodansky, 1935–36; Beard et al., 1931–32, 1939; Borst and Möbius, 1936). Contradictory results were again obtained, and the substitution of gelatin, which is 25 per cent glycine, gave no better agreement (Denis and Minot, 1917).

In connection with other studies on the influence of a high gelatin diet, we followed the excretion of nitrogen, creatinine and creatine in four healthy young men (20–29 years old) who were engaged only in laboratory work during the course of the investigation. Twenty-four hour urines were collected for three consecutive days while on their usual diet. This diet was then supplemented with 60 grams of ossein gelatin daily, 30 in the morning and 30 in the evening. No precise control was exercised over the diet during the gelatin feeding; each man ate as he chose.

Feeding of gelatin continued for 41 days in the case of 3 subjects and for 51 days with the fourth subject, B. C., a colored laboratory helper. Twenty-four hour urines were collected for the first 3 days of each week and excretion studies were continued for at least 7 days following cessation of gelatin feeding. Analyses were performed immediately. Nitrogen was determined by the Kjeldahl method and preformed creatinine by adding alkaline picrate to the fresh urine as described by Folin (1904). For total creatinine the sample was autoclaved with HCl at a temperature of 120° for 30 minutes, and to an aliquot alkaline picrate was also added. Creatine values were obtained by difference. All color comparisons were made in

an Evelyn photocolorimeter. The final values for the creatinine equivalents were obtained from a calibration curve prepared from analyses of pure creatinine solutions.

RESULTS. For convenience, the data are presented graphically (figs. 1 and 2). The added gelatin was equivalent to the daily feeding of 8.8 grams of nitrogen and if its glycine nitrogen were completely converted to creatinine and creatine nitrogen the yield would be about 6 grams (or according to the theory of Beard (1939) the yield would be about 48 grams). The nitrogen excretion of all subjects increased greatly, although in two of the subjects (B. C. and S. M. H.) the extra nitrogen output was less than in the control period when the gelatin nitrogen intake was deducted. Since the subjects were not on a constant diet, we do not know whether nitrogen was retained or the intake of non-gelatin nitrogen was decreased. The excretion of nitrogen in one subject (F. C.) reached 33.5 grams in 24 hours. The nitrogen excretion of three subjects had returned to pre-gelatin values

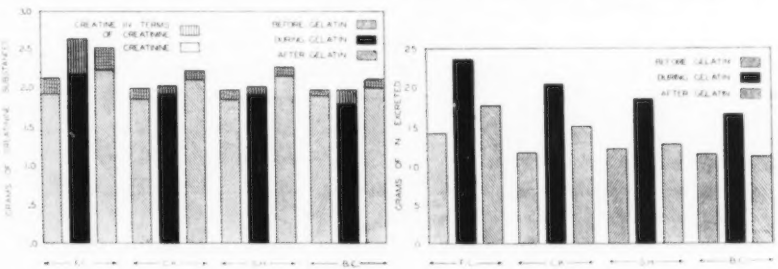

Fig. 1. The average 24-hour excretions of nitrogen, creatinine, and creatine (expressed as creatinine) for four normal men before, during and after a period of gelatin ingestion.

about four days after discontinuing gelatin and in the fourth subject shortly thereafter. (The average values of nitrogen excretion for each subject before, during and after the period of gelatin feeding are shown in fig. 1.)

Although Light and Warren (1934) found no creatine in the urine of males above the age of 19, each of our subjects normally had a measurable creatine excretion even up to 0.27 gram (expressed as creatinine). This is in agreement with Hobson (1939), who recorded creatine excretion in 96 of 97 males. The influence of extra gelatin on the creatine excretion is inconclusive. Two subjects had increases. This was particularly apparent in F. C., who in one 24-hour period excreted over 0.8 gram of creatine (about one-third as much as his creatinine excretion) (fig. 2). In these subjects the increases are unmistakable: in one only 2 of 19 and in the other 2 of 13 urines contained less creatine than in the control periods. However, the other two subjects had extremely variable excretions, and

their average creatine output for the entire period of gelatin ingestion is lower than in control periods. After discontinuing gelatin, the average creatine excretion was slightly above previous control levels. The probability that some creatine had been stored and was being slowly excreted (fig. 2) is indicated by the return of the daily excretions of creatine to pre-

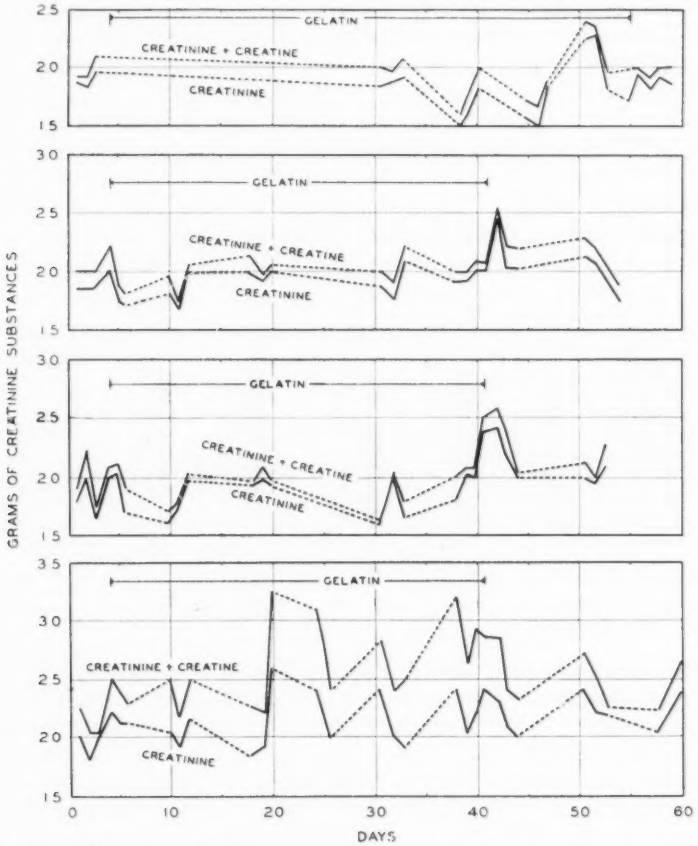

Fig. 2. The daily variations in the excretion of creatinine and creatine of four men before, during, and following the period of gelatin ingestion. (The solid lines indicate days during which the determinations were made.)

gelatin values toward the end of this period. Denis and Minot (1917) were unable to obtain creatinuria in two normal males on a high protein diet which included 50 grams of gelatin. Lewis and Doisy (1918) and Rose et al. (1918) also found no excretion during high protein diets.

As shown by Folin in 1905 and by other workers of that period, the creatinine output in 24 hours is nearly constant for a healthy person and

is uninfluenced by such factors as a high protein, meat free diet. Hobson (1939) states that he was unable to find any significant variation in creatinine excretion of subjects when changed from an adequate but high carbohydrate diet to a low carbohydrate, high protein diet. His mean values for creatinine outputs in 11 subjects are 2.139, 2.140 and 2.107 grams while on a high carbohydrate, and the first and fourth days of a low carbohydrate, high protein diet, respectively. The data presented by us show less regularity in creatinine excretion. The values of creatinine excretion for the four men show an extreme range from minimum to maximum of 0.85 (1.78–2.63); 0.87 (1.57–2.44); 0.82 (1.68–2.50); 0.85 (1.53–2.38) during the entire period of the investigation.

Utilizing average values for creatinine (fig. 1) there is little change in 2 of 4 subjects in its excretion during the period of gelatin ingestion. One of the 4 subjects had a lower average excretion, while another had a much higher excretion. Following the discontinuance of gelatin, the excretion of creatinine was higher in all subjects than in the control periods. Since this was also observed in three subjects during the period of gelatin feeding, the possibility of excretion of exogenous creatinine from stored creatine cannot be entirely ignored. However, the amount actually excreted is small compared to the amount theoretically possible.

Since one-fourth of gelatin is glycine, it is possible that any physiological effects produced by feeding glycine might also be obtained by feeding proportionally larger amounts of gelatin. In treatment of muscle dystrophies gelatin has been found satisfactory by some investigators and unsatisfactory by others. However, gelatin can be effectually substituted for glycine as a detoxifying agent (Griffith, 1934). Denis and Minot (1917) did not observe creatinuria in two males fed 50 grams of gelatin daily. Our data show that creatinuria is possible when subjects ingest 60 grams of gelatin, although it may not necessarily occur to any great extent. This lack of uniform effect is also evident in the feeding of glycine. Borst and Möbius (1936) have failed to observe any influence on the urinary creatine and creatinine of normal adults given 50 grams of glycine. Zwarenstein (1928) also noted no influence (10 grams), while Beard et al. (1939) were able to recover slightly more than the theoretical amount of creatine and creatinine (16.2 grams) formed from the feeding of 5 grams of glycine. Bodansky (1935–36) noted only an effect on creatine output.

We were able to verify Hobson's observation of the presence of creatine excretion in normal adult males. These observations are contrary to general opinion (Folin, etc.). Furthermore, the daily excretion of creatinine is not as consistent as many investigators have reported, although such constancy may be observed in some individuals. As a result, we do not believe in the validity of the assumption that creatinine excretion can be used as a test of the completeness of a 24-hour urinary output (Folin, 1905).

SUMMARY

The addition of 60 grams of gelatin daily to the diet of four males was accompanied by an increased excretion of creatine in two of the subjects. The average excretion of creatinine was not markedly increased by the high protein intake. There was, however, increased excretion of both creatinine and creatine in all subjects on cessation of gelatin ingestion indicating a possible storage during the previous period of gelatin feeding.

In contrast to most previous reports all the subjects normally had some creatine present in their urine and also showed considerable variation in the 24-hour excretions of creatinine.

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ENVIRONMENTAL TEMPERATURES AND THIAMINE REQUIREMENTS[1]

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In this paper will be reported recent studies on optimal thiamine requirements at different levels of environmental temperatures. Previous investigations of vitamin requirements have mostly been carried out without reference to prevailing temperatures or ease of body heat loss. Such disregard of environmental conditions may have little bearing in studies dealing with certain of the vitamins, but for those having to do with tissue combustion processes it seems essential that careful consideration be given to the ease of body heat loss prevailing during the period of experimentation. Tissue combustion rate in normal animals rises as body heat loss is facilitated and falls as difficulty is experienced; the major part of this combustion adaptation takes place during the second and third weeks of acclimatization. It would be expected, therefore, that vitamins serving as catalysts in any phase of the cellular combustion processes would exhibit variation in intake requirement as the combustion rate rises and falls with changes in environmental temperatures.

This we have now found quite sharply true for thiamine. Optimal requirement per gram of food is twice as high at 91°F. as it is at 65°F., while a still higher intake serves to protect against the depressing effects of even more excessive heat. With young rats on diets thoroughly adequate in every way except for thiamine content, signs of inadequacy (lowered food consumption and retarded growth) develop in the hot room at dietary thiamine levels which are entirely adequate for animals in the cold room.

METHODS AND RESULTS. Young Wistar male rats were placed in individual cages in rooms previously described (1), one room being maintained at 65°F. and the other at 91°F. and about 60 per cent relative humidity. Basal diet used was one recommended by Doctor Elvehjem, consisting of

Sucrose	74
Casein, Labco vitamin-free	18
Corn oil	2

[1] The vitamins used in this study were very kindly supplied by Merck & Company, Inc.

Salts[2]	4
Liver extract[3]	2

Riboflavin	1 mgm. per kilo of food
Pyridoxine	2 mgm. per kilo of food
Nicotinic acid	25 mgm. per kilo of food
Choline	300 mgm. per kilo of food
Haliver oil	2 drops per rat per week

TABLE 1

Food consumption, weight gain, and growth efficiency on varied thiamine intake in heat and cold

WEEK ON DIET	GROUP 1			GROUP 2			GROUP 3			GROUP 4			GROUP 5			GROUP 6		
	Food eaten	Weight gain	Grams food / Weight gain	Food eaten	Weight gain	Grams food / Weight gain	Food eaten	Weight gain	Grams food / Weight gain	Food eaten	Weight gain	Grams food / Weight gain	Food eaten	Weight gain	Grams food / Weight gain	Food eaten	Weight gain	Grams food / Weight gain
Cold room, 65°F.																		
First	101	28	3.6	98	31	3.2	100	28	3.5	94	31	3.0	107	33	3.3	90	32	2.8
Second	85	15	5.8	94	23	4.1	119	36	3.3	122	43	2.8	133	43	3.1	117	35	3.3
Third	58	−5		83	11	7.4	128	30	4.3	132	36	3.7	129	32	4.0	127	34	3.8
Fourth	46	−16		75	1	74.5	130	24	5.5	137	32	4.3	138	32	4.3	134	29	4.6
Fifth	50	−10		81	13	6.4	141	25	5.6	138	23	5.9	132	21	6.4	138	24	5.8
Sixth	49	−12		78	7	11.1	132	17	7.7	131	23	5.6	141	25	5.7	131	19	6.8
Hot room, 90–91°F. and 60–70 per cent relative humidity																		
First	54	8	6.7	55	13	4.2	54	12	4.5	54	13	4.2	64	17	3.9	65	20	3.3
Second	37	2	21.3	46	8	6.1	54	16	3.3	56	12	4.6	71	25	2.9	68	25	2.8
Third	22	−2		35	3	12.6	49	14	3.6	54	17	3.3	85	32	2.7	75	28	2.6
Fourth	19	−9		34	2	19.8	47	7	6.4	56	14	4.0	84	23	3.7	75	20	3.8
Fifth	20	−4		34	4	9.7	48	11	4.6	63	19	3.3	79	20	4.0	76	20	3.9
Sixth	20	−3		36	4	10.1	48	10	5.1	60	10	5.8	80	15	5.4	71	14	5.3

Note: Food consumption and weight gain data are given only to the nearest whole gram. The ratios, grams of food/weight gain, were calculated from the actual weighings.

For a rat diet similar to this, Arnold and Elvehjem (2) found 0.8 to 1.0 mgm. thiamine per kilo of ration to be adequate. We therefore added enough thiamine to different batches of the basal diet to give 0.2, 0.4, 0.6, 0.8, 1.2 and 1.6 mgm. per kilo of food mixture. These batches of diet with graduated thiamine content were now fed to groups of rats in

[2] Salt mixture I, as described by Philips and Hart (3).

[3] Liver extract used was Wilson Laboratories' fraction D (70 per cent alcohol-soluble).

the hot and cold rooms, and the weekly food consumption carefully estimated. Detailed data on food consumption and weight gain for the various groups are set forth in table 1.

In figure 1 are shown the marked differences in food consumption by the hot and cold room rat groups. Increase in food consumption in the hot room was almost quantitatively proportional to the graduated increase in thiamine content, up to the fifth group with 1.2 mgm. per kilo of food. Group 6, with 1.6 mgm. per kilo of food, ate slightly less than did group 5.

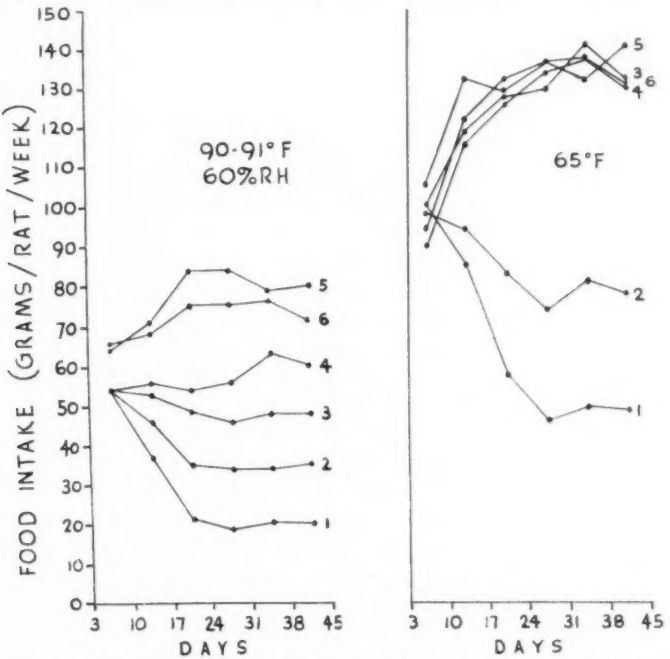

Fig. 1. Dietary thiamine and food consumption in heat and cold. Milligrams thiamine per kilo of food: 1 = 0.2, 2 = 0.4, 3 = 0.6, 4 = 0.8, 5 = 1.2, 6 = 1.6.

In the cold room only groups 1 and 2 exhibited definite thiamine inadequacy in their ability to utilize food. No significant differences were manifested among the four groups receiving the higher thiamine amounts. There was naturally a small amount of food spillage that did not enter into these calculations, but this rarely amounted to more than 1 to 2 grams a week.

Figure 2 presents the growth curves of these same rat groups and shows even more clearly the quantitative response to varying thiamine values in diets of otherwise uniform composition. Group 5 gave best growth performance at 91°F., with group 6 a close second. At 65°F., best growth

was obtained in group 4, with groups 5 and 6 doing consistently less well. In the matter of growth efficiency (i.e., grams of food intake required for each gram of weight gain), best performance was usually given by group 6 in the hot room, but in the cold room by group 4. Everything considered (food consumption, growth rate and growth efficiency), it would seem that

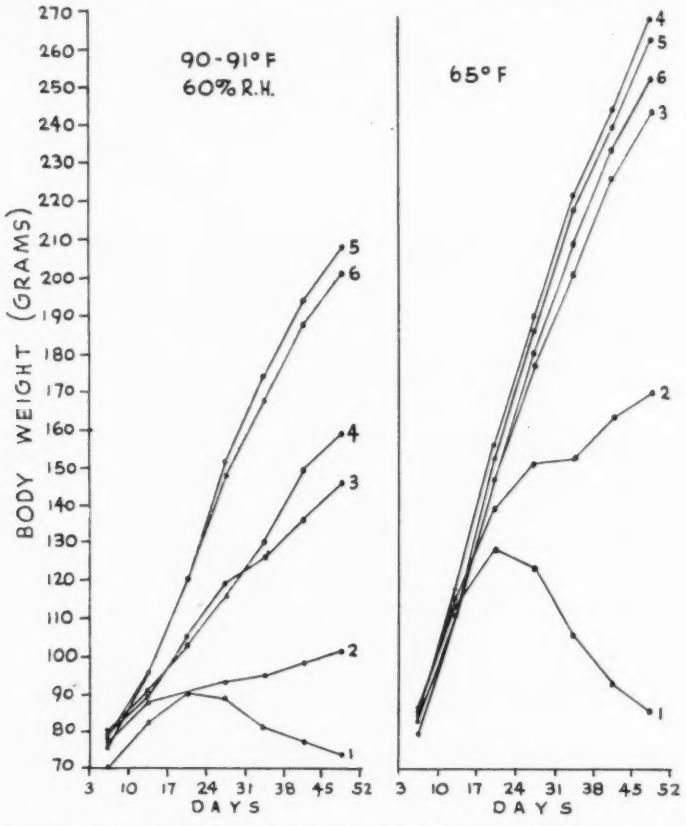

Fig. 2. Dietary thiamine and growth rates in heat and cold. Milligrams thiamine per kilo of food: 1 = 0.2, 2 = 0.4, 3 = 0.6, 4 = 0.8, 5 = 1.2, 6 = 1.6.

optimal response at 91°F. requires just about twice as high a dietary content of thiamine as is needed for best response at 65°F.

Many other workers have described the graduated effect on growth rates and food consumption of increasing dietary thiamine content, but no one seems to have discovered the part environmental temperature level plays in determining thiamine requirements. Waterman and Ammerman

(4) failed to find any definite optimum for thiamine intake in rats, for their rats continued to show growth improvement with progressive thiamine additions up as high as 160 micrograms daily. In our experiments, both at 65 and at 91°F., there is shown a rather clear optimum as concerns dietary thiamine level.

Optimal response in both heat and cold occurred at approximately the same actual thiamine intake. This suggests that the higher dietary content may be needed to keep up the blood and tissue levels at high temperatures because food consumption is reduced. Incidental blood ascorbic acid determinations, made on rabbits kept in the hot and cold rooms and fed a standard diet, gave values only about half as high in hot room animals as in those kept in the cold.

Thus, while there may well exist a definite relationship between thiamine and total non-fat calories of diets used at ordinary laboratory temperatures, this relationship may exist only at higher thiamine levels as difficulty in body heat loss enforces a sharp lowering of food intake and tissue combustion. The findings here presented indicate clearly the need for higher thiamine content in food to be consumed by individuals living under difficult conditions of body heat loss. Growth and eventual adult size in the heat, even at the optimal dietary thiamine level, are always considerably below the corresponding levels of development shown in the cold. Whether this is due to some inadequacy factor other than thiamine at the high temperature levels, or simply to the general suppression of tissue combustion by difficulty in heat loss, cannot yet be said.

Figure 3 shows the protective value of still higher thiamine intake at times when excessive heat is to be encountered. Four rats on the basal diet previously described, to which had been added 0.8 mgm. of thiamine per kilo, were observed at 91°F. for some weeks. The room temperature was then raised to 93°F. for about a week, and the two rats (nos. 4 and 9) showing most marked growth retardation under this heat now had their dietary thiamine doubled. About a month later (67th day, fig. 3) these same two rats began receiving 50 micrograms of thiamine orally each day, in addition to their doubled dietary supply, and at this time the room temperature was raised to 95° or 96°F. and the relative humidity kept at 60 to 70 per cent. Figure 3 shows the rapid down-hill course followed in this severe heat by the rats (nos. 10 and 11) receiving only the normal amount of dietary thiamine (0.8 mgm per kilo of food). Their food intake was reduced to about one-fourth the amount eaten before onset of the severe heat. The rats receiving the doubled dietary and additional oral thiamine on the other hand continued to gain weight in the severe heat; one of them continued his previous rate of daily food consumption and the other actually increased his by 20 per cent.

Rats taken from the 65°F. room and placed directly into the 95 to 96°F.

heat showed less protection from supplemental thiamine administration. All developed hyperpyrexia and lost weight sharply. Weight loss was less, however, with those receiving supplemental thiamine.

Discussion. The need for a higher thiamine content of foods in tropical warmth, or in temperate zone summer heat, has certain important bearings on the problems of human existence. Population masses in regions of tropical warmth, although needing a diet higher in thiamine, actually tend to consume foods of lower thiamine content. Protein foods (meats, nuts, legumes) in general carry a high thiamine content, but they tend to be

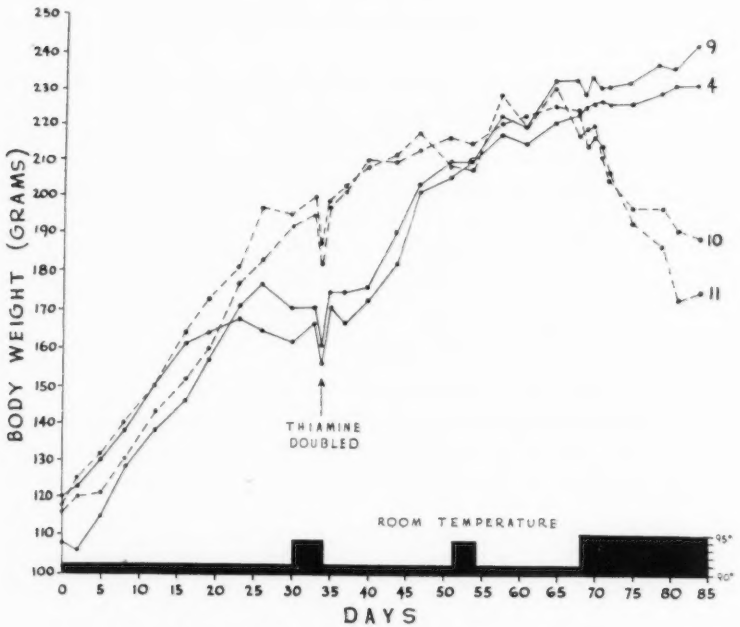

Fig. 3. Thiamine protection against excessive heat

avoided in tropical warmth because of their greater specific dynamic action and higher cost. Instead, people there use more thiamine-poor starchy fruits and tubers. Cereal foods used have usually lost most of their thiamine in preparatory processing. Apparently no amount of thiamine increase could bring tropical residents up to the metabolic level of people living in cooler climates, if we are to judge from the rat response indicated in figures 1 and 2. Such equality could be attained only by supplementary cooling to properly facilitate body heat loss for the tropical residents. But the findings here reported do indicate that a great ad-

vantage would accrue from an adequate thiamine intake even with difficulty in heat loss still persisting.

On an economic basis, it would probably be found less expensive to administer the thiamine directly, in pure form or with some food universally used, than to seek adequate amounts of it in native foods. The peanut affords one of the cheapest and richest natural food sources. Wheat germ and yeast, other rich sources, seem not to be preferred as regular dietary constituents. Lean pork is also high in thiamine, but hogs themselves do poorly in tropical warmth and yield tough, stringy meat.

The strong tendency for a tropical distribution of beri beri, or for its appearance in more northern oriental latitudes during the months of severe monsoon summer heat, is perhaps largely explained by the findings here reported. Similar differences in the requirements for others of the respiratory catalysts may account for the predominance of their associated deficiency syndromes in the lower classes of tropical and sub-tropical populations. The poorer diets consumed by these people do contribute to the production of the deficiency states, but the higher requirement (for thiamine, at least) in tropical warmth greatly exacerbates the effects of any dietary inadequacy that may be present. Since nicotinic acid plays a rôle similar to that of thiamine in cellular combustion, it is quite likely that higher dietary concentrations of this catalyst will also be found advantageous for existence in tropical warmth.

Only by thorough trial can the protective value of thiamine against excessive heat be established for man. Daily administration of supplemental thiamine should make workers in boiler or furnace rooms, or in other types of severe heat exposure, more resistant to the heat effects. It should also prove helpful for temperate zone residents who are hypersensitive to the heat waves of summer and for those who have developed symptoms of heat exhaustion.

CONCLUSIONS

Optimal thiamine requirement for rats (per gram of food or per calorie) is found to be twice as high at 91°F as at 65°F environmental temperature.

Protection against the severe effects of excessive heat is afforded by an accessory thiamine intake above the ordinary daily need.

Discussion is offered of the bearing these facts may have in regard to the problems of human existence under conditions of depressive heat.

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THE EFFECT OF EMOTION, SHAM RAGE AND HYPOTHALAMIC STIMULATION ON THE VAGO-INSULIN SYSTEM[1]

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Through the important studies of Cannon we are well informed about the excitation of the sympathetico-adrenal system under conditions of emotion. The fact that severe emotional disturbances (fear, terror) may be accompanied by excitation of certain branches of the parasympathetic system as well as sympathetico-adrenal discharges, was not unknown to Cannon, but he assumed that it represented a pathological phenomenon in which the reciprocal relationship between sympathetic and parasympathetic innervation is disturbed rather than the expression of a physiological mechanism characteristic of the emotional process. It cannot be doubted on the basis of clinical experience that parasympathetic discharges may occur even in relatively mild states of emotional excitement. For example, weeping is brought about as a result of parasympathetic excitation (Lund). Excited emotion may cause increased gastric secretion (Wittkower) or may precipitate a biliary colic (Bergmann). Increased peristalsis and more frequent urge to urinate are common signs of increased parasympathetic activity during emotional excitement. Startle may produce a marked fall in pulse rate (Tomaszewski). These findings seem to indicate that the emotional process is characterized by discharges affecting both branches of the autonomic nervous system at the same time.

The results of electrical stimulation of the hypothalamus also lend support to this idea, since in contrast to older observations of Ranson, Kabat and Magoun, signs of parasympathetic excitation may be elicited from the whole hypothalamic area provided that weak stimuli or stimuli of low frequencies are used (Masserman and Haertig; Hare and Geohagan). Our own experiences (Carlson, Gellhorn and Darrow) indicate that hypothalamic stimulation may lead to excitation of the parasympathetic and the sympathetic, and also to an inhibition of the parasympathetic, and that excitation of both systems may result from the stimulation of the

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same part of the hypothalamus. On the basis of these experiments it is not impossible that hypothalamic stimulation induced either by electrical stimulation or under conditions of emotion can lead to both parasympathetic and sympathetic discharges.

Feldman, Cortell and Gellhorn (1940) were able to show recently that chemical stimulation of autonomic centers leads to a simultaneous discharge over both the sympathetico-adrenal and the vago-insulin systems. It was deemed of interest to investigate whether the processes of sham rage as induced by hypothalamic stimulation or of rage and other forms of emotion in the waking animal may also involve the vago-insulin system.

Methods. The experiments which were carried out on cats may be divided into three groups. In the first group the animals were anesthetized with chloralosane (100 mgm./kgm. subcutaneously). The adrenals were removed and the liver denervated in order to eliminate the effect of hypothalamic stimulation on part of the sympathetico-adrenal system. The hypothalamus was stimulated with faradic currents and a typical sham rage reaction was produced. The Horsley-Clarke apparatus was used to place the electrode into the hypothalamus. In order to evaluate the effect of sham rage on the vago-insulin system, blood samples were taken before and after bilateral vagotomy at various intervals and analyzed for sugar by the Somogyi modification of the Shaeffer-Hartman method.

In the second group of experiments the spinal cord was sectioned at the sixth cervical segment and the thyroid and parathyroid glands were removed under ether. Eighteen hours later the animals were lightly anesthetized with chloralosane (35 mgm./kgm. intravenously) and the hypothalamus was stimulated as in the first group.

In a third group the cervical cord was sectioned as in the second group and 18 hours later the cat was confronted with a barking dog in an attempt to elicit a rage response. This experiment was repeated after the vagi had been cut subdiaphragmatically without the use of narcosis.

Another group of experiments was performed on rats which were divided into three groups (normals, adreno-demedullated rats and adreno-demedullated rats which had been vagotomized below the diaphragm). These rats were subjected to "emotional excitement" by exposing them to the noise of fire-crackers, which has been found to stimulate the sympathetico-adrenal system in normal rats (Harris and Ingle). Furthermore, the influence of struggle resulting from tying the animals to a board and from the application of slight faradic shocks was also studied on the vago-insulin system (Lumley and Nice).

Results. Figure 1 illustrates two experiments typical for the first group in which the effect of a hypothalamic stimulation on the blood sugar was studied in animals deprived of the adrenals, the thyroid and parathyroid glands and in which the liver had been denervated. In the first

experiment (1) the blood sugar was constant at a very low level. Stimulation of the hypothalamus immediately posterior to the mammillary body elicited a marked sham rage response with arching of the back, extension of the forelimbs and an excellent pilomotor response. The blood sugar was slightly decreased during the half-hour which followed the period of excitation. Hereafter the vagi were cut and the period of stimulation was repeated, giving rise to a response qualitatively and quantitively similar to that observed prior to vagotomy. But the blood sugar response is entirely different. Instead of observing a fall in blood sugar a temporary

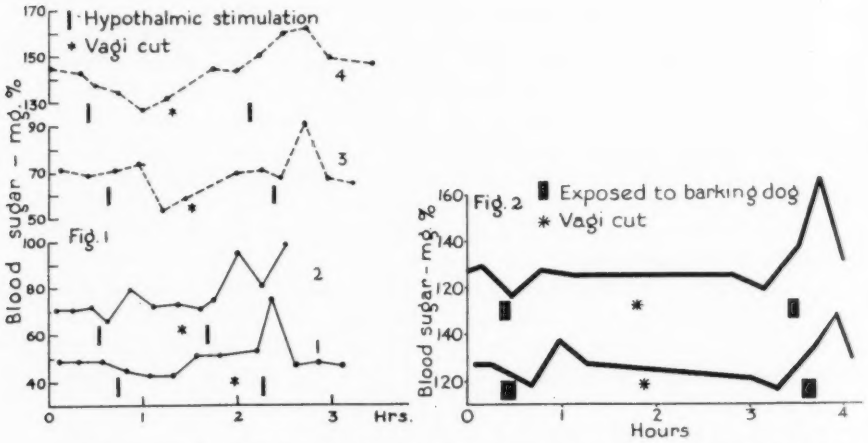

Fig. 1. The effect of hypothalamic faradic stimulation (indicated by the black rectangle) on the blood sugar of cats before and after vagotomy. Vagi were cut at *. In the experiments of graphs 1 and 2 the adrenals were removed and the liver denervated. In graphs 3 and 4 the cervical spinal cord of the cats had been sectioned at the 6th cervical segment 18 hours prior to the experiment.

Fig. 2. The effect of rage on the blood sugar of cats whose spinal cord had been sectioned at the 6th cervical segment before and after vagotomy.

rise occurred. The second experiment, in which the mammillary body was stimulated, showed no marked alteration in blood sugar following the sham rage reaction in spite of distinct signs of pilomotor reactions. It is found that the blood sugar decreased slightly at first and then showed a small rise. However, after the vagi had been cut the blood sugar rose distinctly and for a long time.

All experiments of this group have one feature in common, i.e., that the rise in blood sugar after vagotomy is much more marked in these animals with inactivated adrenals than it is when the vagi are intact. The nature of this blood sugar rise is not fully understood. It is probable that in this

group of experiments sympathin plays a rôle. It is also conceivable that the liver was not completely denervated in every instance and that some effect of stimulation of sympathetic fibers leading to the liver was not eliminated. However, this interpretation is not very likely since Britton found no or only a very slight influence on glycogenolysis of the sympathetic fibers innervating the liver under conditions of emotional excitement (Cannon). It seems to us of greater importance to show that the vagotomy invariably altered the blood sugar response to hypothalamic stimulation, and that the effects indicate that hypothalamic stimulation leading to the characteristic syndrome of sham rage produces hypoglycemia via the vagi which may or may not be overshadowed by the simultaneous liberation of sympathin.

In the second group the sympathetico-adrenal system was eliminated by sectioning the cord at the sixth cervical level. In addition, the thyroid and parathyroid glands had been removed. The effects of sham rage on the blood sugar were quite similar to the first group. Graph 3 of figure 1 shows that stimulation of the lateral hypothalamic area leading to a mild sham rage reaction characterized by spreading of the claws and pupillary dilatation caused a fall in blood sugar when the vagi were intact. This effect was completely reversible. If, however, the stimulation was repeated after vagotomy, the only effect was a temporary rise in blood sugar. Graph 4 represents a similar experiment in which the lateral hypothalamic area was stimulated. The blood sugar level was high but remained constant over many hours. Following the first period of hypothalamic stimulation in which the vagi were intact a distinct fall in blood sugar was observed. Eighty minutes after the period of stimulation the blood sugar had returned to the original level. The experiment was then repeated after bilateral vagotomy and led now to a distinct rise in blood sugar. Another experiment was also characterized by a relatively high blood sugar level but this level was maintained throughout the experiment quite satisfactorily. The electrode was inserted close to the red nucleus. A marked rage reaction was observed, with dilatation of the pupil, increased respiration, upward movements of the forelimbs and swallowing. The stimulation produced a distinct fall in blood sugar which was reversible within 15 minutes. After vagotomy a similar period of stimulation producing a rage reaction closely akin to the reaction observed during the first test resulted in a distinct and reversible rise in blood sugar.

All the experiments show convincingly that hypothalamic stimulation leading to the syndrome of sham rage produces in cordotomized and thyroidectomized animals a fall in blood sugar which is mediated by the vagi. After these nerves have been divided, a slight rise results from the hypothalamic stimulation.

It seems to be of interest to discuss briefly an apparent exception to this

rule. In an experiment in which the animal was prepared as in the last group and in which the mammillary peduncle was stimulated, the blood sugar fell 34.4 mgm. per cent as a result of hypothalamic stimulation. Because the animal was used for another experiment involving the nictitating membrane, the vagi were then cut but the sympathetic, which can easily be separated from the vagi in the cat, was left intact. In this experiment it was observed that after vagotomy a less but still distinct fall in blood sugar (22.6 mgm. per cent) was observed. It seems likely in the light of the other experiments discussed in this paper, in which such hypoglycemic effects after transection of the vago-sympathetic were not observed, that the "sympathetic" may contain some vagal fibers sufficient to increase insulin secretion.

The last group of experiments on cats comprises those experiments which were performed on cordotomized cats without anesthesia. The animals were confronted with a barking dog and in some instances in which they did not react satisfactorily to the dog they were teased for several minutes. The effect of the rage thus elicited was studied on the blood sugar before and after the vagi had been cut below the diaphragm as illustrated in figure 2. The upper graph of this figure shows a relatively high blood sugar level at the beginning of the experiment. The rage reaction induced a fall in blood sugar which was to a large extent reversible. Abdominal vagotomy was performed and several hours later a second rage reaction was elicited. The effect was quite different from that observed in the first test since now a marked rise in the blood sugar resulted. The experiment illustrated in the lower graph of figure 2 shows only a slight variation in blood sugar after the rage reaction but a marked rise resulted when the experiment was repeated after division of the vagi below the diaphragm.

The experiments seem to show that sham rage produced by hypothalamic stimulation and rage elicited in the cat by confronting it with a barking dog result in a hypoglycemic effect which is due to excitation of abdominal branches of the vagi. This interpretation is strengthened by the experiments of Britton and La Barre, who showed that stimulation of abdominal vagal fibers leads to hypoglycemia by increasing the rate of secretion of insulin.

Rats subjected to the noise of fire-crackers for three minutes show typical motor responses suggesting fear. The effect on the autonomic centers is similar to that resulting from the injection of metrazol as reported by Feldman, Cortell and Gellhorn. Normal rats respond to the stimulus with a considerable hyperglycemia whereas adreno-demedullated rats show a temporary fall in blood sugar averaging 20 mgm. per cent. Fifteen minutes later the sugar level has returned to approximately the control value. Rats in which in addition to the demedullation of the adrenals the vagi have been cut below the diaphragm show a rise in blood sugar of a

few milligrams per cent which in spite of its statistical significance is hardly significant physiologically (table 1).

TABLE 1

Effect of fear on blood sugar*

RAT NO.	BLOOD SUGAR (MGM. PER CENT)		
	Control	Time after stimulation	
		1-5 min.	15-20 min.
A. Normal rats			
1	70.9	96.8	81.1
2	73.1	97.8	86.0
3	74.1	102.1	86.0
4	73.1	98.9	91.3
5	75.2	102.1	97.8
6	70.9	94.6	86.0
Mean	72.9	98.7	88.0
Standard dev.	1.6	2.8	5.3
P		<0.01	<0.01
B. Adrenalectomized rats			
1	65.5	43.0	64.5
2	66.6	40.8	65.5
3	63.4	51.6	64.5
4	65.5	43.0	59.1
5	64.5	49.4	60.2
6	67.7	46.2	56.9
Mean	65.5	45.7	61.8
Standard dev.	1.4	3.8	3.2
P		<0.01	0.034
C. Adrenalectomized-vagotomized rats			
1	68.8	68.8	76.3
2	65.5	70.9	73.1
3	67.7	70.9	77.4
4	66.6	69.8	73.1
5	66.6	68.8	82.7
6	68.8	70.9	75.2
Mean	67.3	70.0	76.3
Standard dev.	1.2	0.9	3.3
P		<0.01	<0.01

* The rats were exposed to the noise of fire-crackers for 3 minutes.

A final series of experiments was conducted on rats which struggled because they were tied to a board. The struggle was reinforced by ap-

plication of painful stimuli (faradic shock) to the toes. The results reproduced in table 2 are similar to those obtained in experiments involving fear reactions.

In both sets of experiments it was found that rats subjected to emotional

TABLE 2

Effect of struggle on blood sugar*

RAT NO.	BLOOD SUGAR (MGM. PER CENT)		
	Control	Time after completion of stimulus	
		3 min.	15 min.
A. Normal rats			
1	74.1	95.6	79.5
2	77.4	97.8	84.9
3	75.2	99.9	79.5
4	72.0	89.2	75.2
Mean	74.7	95.6	79.8
St. dev.	1.9	4.0	3.4
P		<0.01	0.044
B. Adrenalectomized rats			
1	65.5	51.6	64.5
2	67.7	48.3	60.2
3	63.4	47.3	64.5
4	63.4	54.8	58.0
Mean	65.0	50.5	61.8
St. dev.	1.8	2.9	2.8
P		<0.01	0.104
C. Adrenalectomized-vagotomized rats			
1	64.5	70.2	74.1
2	66.6	68.8	70.2
3	64.5	68.8	68.8
4	66.6	70.2	68.8
Mean	65.6	69.5	70.5
St. dev.	1.1	0.7	2.2
P		<0.01	<0.01

* The rats were tied to a board for 10 min.

excitement react with a marked hyperglycemia when the adrenals are intact and with a conspicuous hypoglycemia after demedullation of the adrenals. Since the latter reaction disappears after the additional sectioning of the vagi below the diaphragm it is evident that the hypoglycemic reaction elicited by emotional excitement in adreno-demedullated animals

is due to an increase in the rate of insulin secretion brought about by central stimulation of the vagus.

Discussion. The experiments reported in this paper show clearly that hypothalamic stimulation leading to the sham rage syndrome is accompanied by a fall in blood sugar in animals in which the secretion of adrenalin by sympathetic excitation is eliminated. This fall seems to be due to impulses reaching the pancreas via the vagi since the reaction is abolished when the vagi are cut and the experiment is repeated with the same outward success. The fall in blood sugar is slight and occasionally absent, but the comparison of the blood sugar reaction obtained in the normal and the vagotomized animal invariably indicates a greater hyperglycemic effect of the sham rage reaction in the latter. Since it is well known that hypothalamic stimulation (Magoun and collaborators) as well as emotional excitation (Bodo and Benaglia; Partington) may lead to the contraction of the denervated nictitating membrane in adrenalectomized animals, it is highly probable that the rise in blood sugar observed in adrenalectomized and vagotomized animals is due to sympathin. The fact that these results were obtained in animals with the thyroid and parathyroid removed clearly proves that any alteration in thyroid secretion is not responsible for changes in the blood sugar level.

Since the identity of the autonomic changes accompanying sham rage and rage reactions may be questioned, it is of great importance to decide whether the results obtained in experiments on sham rage are applicable to the natural emotional process. The rage reaction elicited in the cat by a barking dog was chosen for the study of the autonomic changes in emotion. This reaction is known to produce marked sympathetico-adrenal discharges (Cannon). When the effect on the sympathetico-adrenal system had been eliminated by the sectioning of the spinal cord at the sixth cervical level, it was found that rage produced a hypoglycemic reaction mediated by the vagi but such reaction was absent after the vagi had been sectioned below the diaphragm.

This work was confirmed and extended by experiments on rats involving struggle and, in another group, "emotional excitement" by exposure to loud noises. Since in the rat experiments the sympathin response is apparently very small, the effect on the vago-insulin system as shown by the hypoglycemic response to excitement is still more distinct than in our experiments on cats.

It is interesting to note that the hypoglycemic response to noise of adrenalectomized rats was observed by Harris and Ingle. Lumley and Nice found in the majority of their adrenalectomized rats a hypoglycemic response to struggle and Britton found the rage reaction to cause a fall in blood sugar in adreno-demedullated cats. However, these authors failed to see the significance of their findings and did not study the effect of

abdominal vagotomy which disclosed the nature of the hypoglycemic reaction.

Our experiments explain also an apparent paradox observed by Bodo and Benaglia. These authors found that both stimulation of the accelerator nerves and emotional excitement cause a similar contraction of the denervated nictitating membrane in cats with inactivated adrenals. However, the blood sugar rose 100 to 200 mgm. per cent in the case of the accelerator nerve stimulation whereas in conditions of emotional excitement the rise in blood sugar was very slight. On the basis of our experiments it must be assumed that emotional excitement caused the liberation of insulin as well as sympathin. The effect on the blood sugar is consequently less than it is in the case of stimulation of the accelerator nerves although the amount of sympathin liberated and tested by the nictitating membrane may be similar in both instances.

The experiments show also that various forms of emotional excitement (fear, rage) although calling forth different cerebrospinal responses (motor patterns) act in a similar manner on the vago-insulin and the sympathetico-adrenal systems.

Cannon has repeatedly emphasized the great physiological significance of the increased blood sugar level for conditions of fighting which accompany emotional excitement. If we consider the blood sugar level alone, the activation of both vago-insulin and sympathetico-adrenal systems might be looked upon as a disadvantageous reaction, since the insulin secretion must have a tendency to counteract the rise in blood sugar. It must be remembered, however, that the utilization of glucose depends not only on the blood sugar level but also on the amount of insulin present (Soskin). Consequently a hyperglycemic reaction combined with increased insulin secretion creates optimal conditions for the utilization of glucose. The vago-insulin and the sympathetico-adrenal system act as synergists as far as utilization of glucose is concerned. This synergistic action is made possible by the greater reactivity of the sympathetico-adrenal system which causes and maintains a hyperglycemia in spite of the increased secretion of insulin under conditions of emotional excitement. It is interesting to note that this relationship is preserved under various conditions leading to a central excitation of the autonomic centers, since similar results are obtained in anoxia, after metrazol (Feldman, Cortell and Gellhorn), and electrically induced convulsions (Kessler and Gellhorn), and under the influence of cocaine and bulbocapnine (Feldman, Cortell and Gellhorn).

SUMMARY

If the effect of central excitation on the adrenal system is eliminated (denervation of adrenals, sectioning of the spinal cord), it is found that

sham rage produced by faradic excitation of the hypothalamus is accompanied by a fall in blood sugar. Since this effect is abolished by subdiaphragmatic vagotomy it is assumed that the hypoglycemia is a result of excitation of the vago-insulin system.

The rage reaction causes a fall in blood sugar in cats in which the cervical spinal cord has been sectioned. After vagotomy, rage produces in such animals a slight hyperglycemia which is probably due to the action of sympathin.

Fear and struggle cause hypoglycemia in adreno-demedullated rats and no change or a slight rise in the blood sugar (sympathin) in adreno-demedullated-vagotomized rats. Since in normal animals emotional excitation (fear, rage) and sham rage cause hyperglycemia it follows that emotion as well as sham rage causes a discharge over both vago-insulin and sympathetico-adrenal systems with a predominance of the latter. The significance of this phenomenon is discussed.

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THE COMPOSITION OF GASTRIC JUICE AS A FUNCTION OF THE RATE OF SECRETION

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It was observed in a previous study (1) that as the rate of secretion of gastric juice increases, the *output* of neutral chloride increases, whereas the *concentration* of neutral chloride diminishes to a limiting value of approximately 11 m.eq./l. There are two possible explanations for this observation: *a*, the parietal cell may secrete neutral chloride, or *b*, increased parietal cell activity may be accompanied by a corresponding increase in the secretion of neutral chloride by non-parietal cells. A decision regarding the relative importance of these alternative explanations must precede the formulation of any theory to account for the variations in the composition of gastric juice.

A study of the behavior of the individual cations which compose the neutral chloride of gastric juice was accordingly undertaken, since this appeared to provide the most direct approach to the problem. In addition the possible influence of the rate of secretion on the osmotic pressure of gastric juice was investigated. The ultimate purpose of this study was to seek an explanation for the variations in the composition of gastric juice.

METHODS. For this study 183 samples of gastric juice were collected on two successive days from six dogs with vagotomized pouches of the entire stomach, in which a continuous flow of gastric juice was maintained by repeated injections of histamine at 10 minute intervals. These samples, collected at 20 minute intervals, were pooled at the time of collection according to the volume-rate of secretion to form nine pooled samples. These were then subjected to chemical analysis and the data thus obtained were subjected to a thorough statistical analysis. The details of the physiological procedures and the statistical methods are given in a previous paper (1).

The total acidity was determined by titration using phenolphthalein as the indicator. Total chlorides were determined by Volhard titration following Na_2CO_3 fusion. Neutral chloride was calculated by difference. Calcium and potassium were determined by the methods of Clark and Collip (2) and Breh and Gaebler (3) respectively, using neutralized aliquots.

Sodium was calculated by subtracting from the neutral chloride the sum of the potassium and calcium. Freezing point depressions were measured with a Beckmann thermometer.

RESULTS. The results of the chemical analyses of the 9 pooled samples of gastric juice are presented in table 1. Attempts to detect fatty acids in these samples were unsuccessful because of their extremely low concentration. This is in contrast to the report of Ling, Liu and Lim (4).

Relationships between output and rate of secretion. The outputs of total, acid, and neutral chloride, K, Na, Ca, and total osmotic units (arbitrarily expressed as the product of volume and freezing point depression in degrees Centigrade) bear in each case linear relationships to the volume-rate of secretion. With the exception of the case of sodium, the correlation coefficients are 0.9 or greater, indicating a high degree of correlation, and the

TABLE 1

Chemical composition of 9 pooled samples of gastric juice

VOL./20 MIN.	Cl	HCl	BCl	Na	K	Ca	OSMOTIC PRESSURE
cc.	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*°C.*
1 6.0	155.1	93.5	61.6	52.8	7.4	1.350	0.572
2 8.0	157.3	110.7	46.6	38.4	7.2	0.952	0.588
3 9.7	159.0	121.0	38.0	30.0	7.2	0.760	0.591
4 12.5	161.0	120.0	41.0	32.8	7.4	0.800	0.596
5 15.2	162.1	130.4	31.7	23.6	7.4	0.654	0.602
6 18.3	161.0	134.0	27.0	19.5	7.0	0.544	0.600
7 21.2	164.2	138.0	26.2	18.5	7.2	0.530	0.609
8 24.3	162.6	137.0	25.6	17.7	7.4	0.482	0.604
9 29.1	162.6	142.0	20.6	12.8	7.4	0.400	0.605

slopes (b constants) are significantly positive in a statistical sense, indicating that the outputs increase with the rate of secretion (see table 2).

Relationships between concentration and rate of secretion. The concentrations of total, acid, and neutral chloride, Na, Ca, and osmotic pressure bear hyperbolic relationships to the rate of secretion. In every case the correlation coefficients are greater than 0.9 (see table 3). The hyperbolic relationships are direct in the case of total and acid chloride, and osmotic pressure, indicating maximal asymptotic levels at rapid rates of secretion. Thus, as revealed by the d constants, the maximal concentration of total chloride is 165.6 m.eq./l., of acid chloride, 153.1 m.eq./l., and of freezing point depression, 0.617 degree C.

The hyperbolic relationships are inverse in the case of neutral chloride, Na and Ca, indicating asymptotic minimal levels at rapid rates of secretion. Thus, as revealed by the d constants, the minimal concentration of neutral chloride is 12.5, of Na, 5.1, and of Ca, 0.2 m.eq./l. In the case of sodium the figure is not statistically significant.

TABLE 2

Relationships between volume-rate and the outputs of various ions (volume-output method)

	Cl	HCl	BCl	K	Na	Ca	Δ × V
				Correlation coefficients			
	0.999	0.999	0.928	0.999	0.681	0.919	0.999
				Volume independent*			
a	−0.0527	−0.3550	0.3009	−0.001229	0.2932	0.006560	−0.02163
b	0.1649	0.1535	0.0115	0.007371	0.004132	0.0001977	0.6139
σ_b	0.000880	0.00142	0.00175	0.000118	0.00168	0.0000321	0.00328
S_X	0.0172	0.0277	0.0341	0.00231	0.0328	0.000626	0.00640
				Volume dependent†			
a′	0.3336	2.1800	−23.13	0.1954	−24.28	−25.51	0.3550
b′	6.0590	6.5780	80.73	135.42	112.15	4269.55	1.6285
$\sigma_{b'}$	0.0324	0.0610	12.29	2.17	45.62	693.3	0.00871
S_Y	0.104	0.181	2.76	0.313	5.40	2.91	0.104

* The regression of Cl, HCl, BCl, K, Na, Ca, and Δ (designated by X) on volume-rate (V) has the following form:

$$X = a + bV$$

The standard error of estimate of X is denoted by S_X and the standard error of b, the slope of the line, by σ_b.

† The regression of V on X is given by $V = a' + b'X$ and the standard errors of estimate are designated similarly to the above.

TABLE 3

Relationship between volume-rate and concentration of various ions (volume-concentration method)

Volume independent*

	[Cl]	[HCl]	[BCl]	[Na]	[Ca]	Δ
			Correlation index			
	0.955	0.987	0.978	0.978	0.979	0.945
c	−62.98	−349.36	286.38	278.91	6.509	−0.2568
d	165.57	153.05	12.52	5.093	0.1999	0.6168
σ_d	0.72	1.93	2.06	2.01	0.0470	0.00286
$S_{[X]}$	0.88	2.36	2.52	2.46	0.0575	0.00350

* The hyperbolic regression of [Cl], [HCl], [BCl], [Na], [Ca], and Δ (designated by [X]) on V, is given by:

$$[X] = \frac{c}{V} + d.$$

In contrast to the above, the concentration of K remains constant at an average level of 7.4 m.eq./l., in spite of wide fluctuations in the rate

of secretion and in the acidity. This unique constancy of the K ion has been noted by others, not only in gastric juice (5, 6, 7, 8), but in saliva (9, 10).

The systematic variation in osmotic pressure was somewhat unexpected, since both mucous secretion (11) and highly acid gastric juice (12) have been reported to be isotonic. In order to investigate this phenomenon further, we determined the osmotic pressure of *a*, basal gastric secretion which failed to turn litmus paper red; *b*, slightly acid gastric juice secreted slowly in response to minimal doses of histamine, and *c*, highly acid juice secreted rapidly in response to large doses of histamine. As shown in table 4, the non-acid samples had fairly high osmotic pressures (average 0.597), whereas the faintly acid samples had lower (0.575) and the highly acid samples higher (0.610) osmotic pressures. The simplest explanation for these observations is that the interaction of the bicarbonate of the isotonic mucous secretion with the acid of the isotonic acid secretion

TABLE 4

Relationship between osmotic pressure and rate of secretion

DOG	NON-ACID		SLIGHTLY ACID		HIGHLY ACID	
	cc.	Δ	cc./20 min.	Δ	cc./20 min.	Δ
1	28.3	0.580	3.2	0.570	15.8	0.620
2	15.5	0.590	3.1	0.580	9.8	0.590
3	45.4	0.600	5.0	0.570	27.6	0.610
4	9.8	0.618	3.7	0.580	21.4	0.630
Average.....		0.597	3.7	0.575	18.7	0.612

releases CO_2, which correspondingly reduces the osmotic pressure of the mixture.

Another striking characteristic of the behavior of the osmotic pressure is its close resemblance to that of the total chloride; in fact, when equivalent scales are used, the osmotic pressure hyperbola may be superimposed upon the total chloride hyperbola. This indicates that chloride is the only anion which contributes significantly to the osmotic pressure of acid gastric juice. This fact has been commented upon before in the case of highly acid gastric juice (12).

Relationships between concentrations of various ions. Since the mucous, or non-parietal secretions, are isotonic, their concentration of total base must also be isotonic. A calculation, therefore, of the concentration of other ions corresponding to an isotonic concentration of total base (neutral chloride) would reveal the composition of the non-parietal secretion. Accordingly the relationships between neutral chloride and the other ions were determined. The relationships are in each case linear, with very high correlation coefficients (table 5). The relationship is direct in the

case of Na and Ca, and inverse in the case of total chloride and acid chloride.

Why does the neutral chloride output increase with the secretory rate? The behavior of K in gastric juice is unique in that its concentration remains constant. This can only mean that it is secreted by all the cells of the gastric glands and in the same concentration by each, namely, 7.4 m.eq./l.; if this were not the case, variations in the proportion of parietal secretion in gastric juice would be reflected as changes in K concentration. The increase in the output of potassium which accompanies an increase in the rate of secretion only partially explains, however, the behavior of the

TABLE 5

Relationships between concentration of total base and concentration of other ions (concentration-concentration method)

	[Cl]	[HCl]	[Ca]	[Na]
		Correlation coefficient		
	−0.805	−0.991	0.996	0.999
		BCl independent*		
a	167.50	167.42	−0.08064	−7.0462
b	−0.1968	−1.194	0.02262	0.9726
σ_b	0.0548	0.0627	0.000737	0.00520
$S_{[X]}$	1.52	2.02	0.0238	0.0168
		BCl dependent†		
a′	564.01	138.18	3.800	7.250
b′	−3.293	−0.8214	43.887	1.0280
$\sigma_{b'}$	0.917	0.0431	1.43	0.00550
$S_{[BCl]}$	6.22	1.68	1.05	0.172

* The regression equation has the form [X] = a + b[BCl], where [X] denotes [Cl], [HCl], [Ca], or [Na], and other symbols are used as in previous tables.

† The regression equation with [BCl] as the dependent variable has the form [BCl] = a′ + b′[X].

neutral chloride fraction; the outputs of Na and Ca also apparently increase. Since their concentrations do not remain constant, two possible explanations are available; *a*, they are secreted in very low concentrations (6.0 and 0.21 m.eq./l. respectively) by the parietal cell, or *b*, increased parietal cell activity is accompanied by a slight increase in the activity of non-parietal cells, which alone secrete Na and Ca. The latter explanation is preferred, since the increase in the Na output is questionable from a statistical standpoint and that of the calcium is questionable from an analytical standpoint.

It is very possible that the behavior of Na and Ca would be less ambigu-

ous if gastric juice were collected during sham-feeding, which stimulates non-parietal cells more effectively than histamine.

What is the composition of the parietal secretion? By somewhat different processes of reasoning, Liu *et al.* (13) and Hollander (14) both concluded that the acidity of the parietal secretion is equal to the maximal total chloride concentration of gastric juice. This conclusion is no longer acceptable in view of the demonstration discussed above, that the parietal cell must secrete neutral chloride in the form of a potassium salt.

The first step in predicting the composition of the parietal secretion is to determine the composition of gastric juice which consists as nearly as possible of parietal component only. This condition is met when the juice is being secreted at its maximal rate and at its highest acidity. In

TABLE 6

Limiting values for concentration of ions in gastric juice

ION	ALKALINE SECRETION				MOST ACID SECRETION			
	Conc.-conc. method		Vol.-conc. method	Ave.	Vol.-output method		Vol.-conc. method	Ave.
	BCl ind.	BCl dep.			Vol. ind.	Vol. dep.		
Cl^-	134.8	120.9*	131.8	133.3	164.9	165.0	165.6	165.2
H^+	−30.9	−33.9	−34.2	−33.0	153.5	152.0	153.1	152.9
BCl	134.8	120.9*	131.8	133.3	11.5	12.4	12.5	12.1
B^+	166.0†	166.0†	166.0†	166.0†	11.5	12.4	12.5	12.1
Na^+	154.4	154.4	154.6	154.5	4.1	8.9	5.1	6.0
K^+				7.4	7.4	7.4	7.3	7.4
Ca^{++}	3.67	3.70	3.69	3.69	0.20	0.23	0.20	0.21
HCO_3^-	30.9	33.9	34.2	33.0				
Δ	0.615†	0.615†	0.615†	0.615†	0.614	0.614	0.617	0.615

* Value not included in average.

† Value assumed to make secretion isotonic.

table 6 the limiting values for the various ions corresponding to an infinite rate of secretion are presented. These figures must be modified, however, for we have concluded that the small amounts of Na and Ca which are present in the most acid juice have been contributed by non-parietal cells. After taking this factor into consideration, the composition of parietal secretion, as summarized in table 7, is estimated to be 166 m.eq./l. of Cl, 7.4 of K, and the difference, 158.6, of hydrogen ion.

What is the composition of the non-parietal secretion? Since it has not been possible in the present investigation to differentiate the secretory products of the peptic, mucous chief cells, and the surface epithelium, the term non-parietal secretion is used to refer collectively to their contribution to gastric juice.

In regard to K, it has already been shown that it must be secreted by the non-parietal cells in a concentration of 7.4 m.eq./l. In regard to the other ions a less direct method of estimation must be employed. It has been demonstrated that the non-parietal secretion is isotonic, and hence its concentration of total base must be isotonic, namely, 166 m.eq./l. From the equations which relate the concentration of neutral chloride (or total base) to the concentrations of Na, Ca, total, and acid chloride, one can calculate the concentration of the latter ions which correspond to 166 m.eq./l. of total base. The results of such calculations are presented in table 6. It should be noted that the acidity is found to be a negative value, indicating an acid deficit, which has also been entered in the table as bicarbonate.

An analogous calculation can be made from the hyperbolic equations relating the rate of secretion to the concentrations of the various ions. From the neutral chloride (or total base) equation one can derive that a

TABLE 7

Estimated composition of parietal and non-parietal secretions

SECRETION	ANIONS				CATIONS					OSMOTIC PRESSURE
	Chloride		HCO_3	Total	H	Na	K	Ca	Total	
	Total	Neutral								
										°C.
Parietal	166	7.4	0.0	166	158.6	0.0	7.4	0.0	166	0.615
Non-parietal	133	133	33	166	0.0	154.5	7.4	3.7	165.6	0.615

total base concentration of 166 m.eq./l. corresponds to a volume rate of 1.68 cc. per 20 minutes. In the other equations this volume-rate corresponds to the concentration of the other ions in the non-parietal secretion. The results of such calculations are also shown in table 6. The agreement is reasonably close between the estimates obtained by the two methods of calculation.

The values for the bicarbonate ion were determined as acid deficit. The values can be supported, however, by other evidence. Chloride and bicarbonate ions make up nearly the entire anion content of alkaline gastric juice; hence the total base of 166 minus the chloride of 134.8, gives 31.2 m.eq./l. for the bicarbonate fraction, a reasonable check. Furthermore, since the osmotic pressure so closely follows the total chloride concentration, the fall in osmotic pressure which accompanies the mixing of parietal with non-parietal secretion is exactly predictable on the basis of CO_2 evolution.

The averaged values which represent the estimated composition of the

non-parietal secretion are presented in table 7. These values are within the ranges reported by other investigators who used other methods (see Hollander, 15).

Discussion. *What is the explanation for the variations in the composition of gastric juice?* If gastric juice is a mixture of two components, the parietal and the non-parietal, then the composition of gastric juice can vary only within the limits set by the composition of these two components. Under conditions where there is no parietal cell activity, the gastric juice will take on the composition of the non-parietal secretion; when parietal cell activity greatly predominates, the juice will approach the composition of the parietal secretion.

As a result of the present analysis of gastric juice secreted in response to histamine, it can be said that the non-parietal component remains relatively constant in its rate of secretion. In the particular dogs used, the rate averaged 1.68 cc. per twenty minutes. It may increase very slightly under the influence of histamine, and would very likely increase significantly under the influence of sham-feeding or the ingestion of a meal. The rate of secretion of the parietal component, on the other hand, depends upon the strength of the stimulus. In the particular dogs used, the maximal rate attained was approximately 26 cc. per 20 minutes. Since the composition of gastric juice depends upon the relative proportions of its two components, and since one of these components is small in quantity and constant in its rate of secretion, whereas the other varies widely, it must be concluded that *the composition of gastric juice is a function of its rate of secretion.*

It should be pointed out that this principle does not demand that all samples of gastric juice of a given volume-rate have identical composition. Obviously, different individuals, or the same individual at different times may exhibit differential rates of secretion of the two components so that the proportion in a given total volume need not be always the same. Nevertheless, even in these cases the composition of the juice will be a function of its rate of secretion. The only exceptions to this generalization would be *a*, when a secretory stimulus is equally effective in activating the two component secretions, or *b*, when only one of the two components can be formed by the gastric glands.

An apparent inconsistency with this generalization is the observation that at equal rates of secretion the acidity is usually lower during the ascending than during the descending portion of a secretory curve obtained following the injection of histamine. This is probably related to the fact that the meagre and viscous non-parietal secretion tends to accumulate in the lumen of the stomach and its glands and is "washed out" in excess during the first portion of the response to histamine. This complicating

factor was, of course, avoided in the present investigation by collecting a continuous secretion, and then only after the first hour's samples had been discarded.

CONCLUSIONS

1. The outputs of HCl, Cl, BCl, K, Na, and Ca by the gastric glands increase with the rate of secretion in linear fashion, but at different rates.

2. The concentrations in gastric juice of BCl, Na, and Ca decrease, and the concentrations of Cl, HCl, and the osmotic pressure increase with the rate of secretion in hyperbolic fashion, but at different rates.

3. The concentration of K remains uniquely constant in gastric juice.

4. From these observations it has been possible to show that the parietal cell secretion consists of 166 m.eq./l. of Cl, 7.4 of K, 158.6 of hydrogen ion, and that the non-parietal secretions consist of 133.3 m.eq./l. of Cl, 33.0 of HCO_3, 154.5 of Na, 7.4 of K, and 3.7 of Ca.

5. The composition of gastric juice can vary only between the limits set by its two main components, the parietal and the non-parietal. Since the non-parietal component is secreted at a very slow and practically constant rate, whereas the parietal component is secreted at rates which vary widely with the dosage of histamine, it is concluded that the composition of gastric juice is a function of its rate of secretion.

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REDUCTION OF SEXUAL BEHAVIOR IN MALE GUINEA PIGS BY HYPOTHALAMIC LESIONS[1]

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Lesions in the ventral portion of the hypothalamus of the female guinea pig may bring about disturbances of the reproductive cycle (Dey, Fisher, Berry and Ranson, 1940; Dey, 1941). In addition to the ovarian disturbances which occur in some animals, all animals of the group showed a complete absence of mating behavior and copulatory reflexes. Subsequent studies have suggested that the interference with copulatory behavior is not secondary to ovarian or anterior pituitary hormonal imbalances, but is the result of the destruction of elements of the central nervous system which are necessary to the integration of the behavior pattern (Brookhart, Dey and Ranson, 1940; Brookhart, Dey and Ranson, in press). It is the purpose of this communication to make a report of the effects of similar lesions in the hypothalamus upon the mating behavior of male guinea pigs.

Nine male guinea pigs weighing between 600 and 800 grams were used in this experiment. The lesions were placed in the hypothalamus by means of the Horsley-Clarke instrument bearing a unipolar electrode. In 4 of the animals the lesions had previously been placed by Dr. R. Gaupp in the course of studies on diabetes insipidus and no data on the preoperative sexual behavior are available. The preoperative sexual behavior of the remaining 5 animals was observed by placing them singly in cages with one or more spayed females in induced estrus, and was seen to be normal. Similar behavior tests were made on each animal 2 to 4 weeks postoperatively, each animal being given 3 or more observation periods of at least an hour in duration. In order to obviate the possibility that any deficiency in sexual behavior was due to the strangeness of the surroundings or lack of sexual experience, each of the operated males was placed in a cage with three normal females for a sufficient length of time to allow all of the females to go through two sexual cycles. The presence or lack of pregnancy at the end of the period was regarded as an indication of the sexual potency of the males.

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At the end of the experimental period, which lasted 6 months, each of the animals was subjected to the Batelli (1922) electrical ejaculation test, and the ejaculate was examined for sperm. At the time of sacrifice, smears of the epididymides were examined for motile sperm. Portions of the testes and seminal vesicles were fixed, sectioned and stained with hematoxylin and eosin. The hypothalami were fixed and sectioned, and alternate sections were stained with cresyl violet and Weil stains.

When a normal male guinea pig is placed with receptive females, he begins an immediate round of investigation accompanied by purring, treading, ruffling of the hair on the back of the neck and shoulders, dilatation of the para-anal pouches, and sniffing of the genitalia of the female. A short period of this courtship activity appears to be necessary for the full

TABLE 1

NUMBER	ACTIVITY TESTS	SEMEN	SPERMATOZOA	TESTES	SEMINAL VESICLES	ESTROUS PERIODS	PREGNANCIES
1*	No activity	Scanty	Normal	Normal	Normal	6	0
2*	No activity	Normal	Normal	Normal	Normal	6	0
5	No activity	Normal	Normal	Normal	Normal	5	2
6	No activity	Normal	Normal	Normal	Normal	6	0
7	Treading, purring and sniffing only	Normal	Normal	Normal	Normal	5	1
8	Few mild mountings	Normal	Normal	Normal	Normal	6	0
9	Few mild mountings	Normal	Normal	Normal	Normal	6	0
	Total number of estrous periods—40. Pregnancies—3						
3*	Normal behavior	Normal	Normal	Normal	Normal	5	2
4*	Normal behavior	Normal	Normal	Normal	Normal	5	2
	Total number of estrous periods—10. Pregnancies—4						

* Animals operated by Doctor Gaupp. No preoperative data.

development of sexual excitement. The act of sniffing and rubbing the hair on the back of the female in the wrong direction finally culminates in mounting and copulatory thrusts.

The results of the various observations on the operated males are summarized in table 1. In 2 of the animals which were not operated by us, copulatory behavior was normal. Three of the animals exhibited courtship behavior of varying degrees of intensity, one failing to mount while the other two mounted a few times but executed no copulatory movements. The remaining 4 animals showed absolutely no interest in the estrual females with which they were placed.

Each of the males was caged with 3 normal females throughout the period of 2 full cycles. Depending on whether or not they impregnated

one of the females during the first cycle, each male was with females through 5 or 6 estrous periods and thus had 5 or 6 opportunities to cause pregnancy. Out of a total of 50 opportunities which were offered the 9 males only 7 pregnancies resulted, and no seminal remnants were noted on the genitalia of any of the females which did not become pregnant. The 2 males which showed normal behavior in the preliminary tests accounted for 4 of the pregnancies, impregnation occurring in 4 out of a total of 10 estrous periods. One of the animals which showed mild courtship behavior had 5 opportunities and only impregnated one of the females in his cage. The remaining 2 pregnancies occurred in the case of one of the animals which previously had shown no interest in the females. The remaining 5 animals failed to impregnate any of the females in their cages. Thus, the animals which showed reduced sexual activity while under observation produced only 3 pregnancies out of a total of 40 opportunities.

In all cases but one, the semen which was obtained upon electrical ejaculation was normal in volume and coagulated almost immediately after ejaculation. The seminal vesicles of the one animal which delivered only a scanty amount of semen were seen to be distended with secretion when the animal was autopsied immediately after the attempted ejaculation. The seminal and epididymal smears showed numerous motile sperm in all cases. Microscopic examination of the testes indicated active spermatogenesis and lack of atrophy in the seminiferous tubules. The interstitial tissue was normal in all animals. The seminal vesicle epithelium was high, and negative Golgi images distal to the nuclei were evident in all cases.

With the exceptions of animals 3 and 4, the lesions were similar in location to those already described for the sterile female animals (Dey, 1941). The lesions occurred bilaterally near the ventral border of the hypothalamus between the optic chiasma and the stalk of the pituitary. In the two exceptions which showed normal sexual activity the lesions were similarly located. However, the lesion in one of the animals was unilaterally placed, while that of the other involved only the most superficial portions of the basal surface of the hypothalamus. No significant differences could be noted between the lesions of animals 5 and 7 and the animals which failed to impregnate any of the females in their cages.

The proximity of the lesions in these animals to the anterior pituitary raises the possibility that the reduced sexual behavior was a result of altered anterior pituitary function. However, the immediate postoperative reduction in sexual activity which has been observed argues against the possibility that such a reduction is the result of a lack of testicular hormone, since postpubertal castration causes only a gradual reduction in sexual drive (Ball, 1937; Stone, 1939). In addition, the condition of the seminal vesicle epithelium, the motility of the sperm, and the produc-

tion of a copious coagulable ejaculate in these animals may be taken as an indication of a continued production of the testicular hormone (Moore, 1928; Moore and Gallagher, 1930; Moore, Hughes and Gallagher, 1930). The continuation of active spermatogenesis, the lack of atrophy of the seminiferous tubules, and the continued secretion of testicular hormone, in turn, point to a continuation of normal gonadotropic function on the part of the anterior pituitary. It is therefore suggested that the behavioral deficiency in these animals is the result of destruction of elements of the central nervous system rather than gonadotropic or androgenic hormonal imbalance.

SUMMARY

Lesions which are properly placed in the hypothalamus may abolish or greatly reduce sexual activity of male guinea pigs. This decrease in activity is manifested by a lack of interest in estrous females and by the failure of operated males, with a few exceptions, to impregnate normal estrous females.

The continuation of active spermatogenesis, and the normal maintenance of the seminiferous tubules and of the seminal vesicles indicate normal gonadotropic function on the part of the hypophysis. It is therefore suggested that the behavioral deficiency is the result of a destruction of elements of the central nervous system which are necessary for the mating reactions.

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RIBOFLAVIN DEFICIENCY IN THE DOG[1]

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In a previous publication (1) we described a technique for the rapid production of an uncomplicated riboflavin deficiency in the dog. The present paper records the results of studies made to define more accurately the riboflavin requirement of the growing dog. In addition, the results of *1*, blood riboflavin determinations; *2*, riboflavin "saturation" tests, and *3*, blood chemistry studies during various stages of riboflavin deficiency are reported.

METHODS. *Care of dogs.* Mongrel pups (litter mates), recently weaned, were fed milk for 2 weeks and then placed on the basal ration previously described (1).[3] The ration was supplied *ad libitum*. Daily food consumption records were kept for each dog and the riboflavin supplement, calculated on the basis of the amount of ration consumed the previous day, was given daily by pipette. The riboflavin was administered as an aqueous solution containing 100 micrograms per ml. The dogs were weighed weekly except during critical periods when daily weighings were instituted.

Blood riboflavin determinations. The blood riboflavin analyses were carried out by a microbiological method involving the use of *L. casei* (3, 4). The blood obtained by venepuncture with oxalate as the anticoagulant was hemolyzed in distilled water and levels equivalent to 0.2 and 0.3 ml. of whole blood per assay tube were used. Duplicates were run at each level.

Urinary riboflavin determinations. The dogs were placed in wire-bottom metabolism cages and 24 hour urine samples were collected in dark bottles under toluene. There was no contamination of the urine with fecal mate-

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies were aided by grants from the Rockefeller Foundation, the Wisconsin Alumni Research Foundation and the Works Progress Administration.

[2] Commercial Solvents Corporation Fellow.

[3] The liver concentrate used in these experiments was prepared from liver powder (1–20) or from liver fraction B (the Wilson Laboratories) according to the directions given by Wagner et al (2). The final alkaline irradiation procedure was omitted. The concentrate was fed at a level equivalent to 4 per cent of the original liver extract and contained less than 0.05 microgram of riboflavin per gram of original liver extract when assayed by a microbiological technique (3). We are indebted to Merck and Company, Rahway, New Jersey, for generous supplies of thiamin, nicotinic acid and vitamin B_6.

rial. The 24 hour volume was determined, the pH adjusted to 6.8 and an aliquot was stored in the refrigerator under toluene. When necessary, the urine was diluted with distilled water in order to adjust the riboflavin content to the correct assay range (approximately 0.1 microgram of riboflavin per ml.). All assays were made at 3 levels, each level being run in duplicate. The riboflavin determinations were carried out according to a microbiological method (3, 4).

Blood chemistry determinations. Blood sugar, non-protein nitrogen, urea nitrogen and uric acid determinations were made according to the micromethods of Folin (5) adapted for use in the Evelyn photoelectric colorimeter. Hemoglobin determinations were made periodically.

EXPERIMENTAL. *Nutritional studies.* These studies were performed in an attempt to define the riboflavin requirement of the growing dog. Two animals, dogs I and II, were fed the basal riboflavin-free ration. Other dogs received the basal ration plus varying amounts of synthetic riboflavin.

Dogs I and II exhibited a very poor initial rate of growth and at the end of the third week of the experiment growth had ceased entirely. Various doses of riboflavin were then given at intervals and the resulting growth responses were observed. Some of the responses obtained with dog I are given in figure 1. The growth responses to a given dose of riboflavin were quite consistent for each of the dogs. In this manner both dogs were kept in a chronic state of riboflavin deficiency for 6 weeks. Riboflavin therapy was always followed by a gain in weight. When riboflavin therapy was discontinued, the dogs failed rapidly and died within 2 weeks after the administration of the last dose of riboflavin.

Dogs III and IV received 100 micrograms of riboflavin per 100 grams of ration. A fair rate of growth (370 and 350 grams per week for dogs III and IV, respectively) was observed over a period of 10 weeks. The rapid decline following the removal of the daily riboflavin supplement is noteworthy. Dog III died within 2 weeks after the removal of the riboflavin, as indicated in figure 1, while dog IV failed rapidly and was maintained by the frequent administration of riboflavin. This dog died 7 weeks after the removal of the daily riboflavin supplement.

Dogs V and VI received 200 micrograms of riboflavin per 100 grams of ration. At the end of 7 weeks dog V exhibited the collapse syndrome which is characteristic of acute riboflavin deficiency. The dog was given 400 micrograms of riboflavin per kilogram of body weight by subcutaneous injection and an immediate recovery was effected. Within a week the dog again exhibited the same syndrome and was given 300 micrograms of riboflavin per kilogram of body weight by subcutaneous injection. A temporary recovery resulted but it became necessary to administer a similar dose of riboflavin a few days later. The dog died 8 days after the last treatment. After 9 weeks on experiment (during which the average weekly gain was 350 grams) the daily riboflavin supplement of dog VI was

discontinued. In contrast to the behavior of dogs III and IV, dog VI maintained weight for 3 weeks following the elimination of riboflavin from the diet and 2 additional weeks elapsed before a precipitant loss of weight made it necessary to institute riboflavin therapy. The animal was in good condition at this time. Growth responses to various doses of riboflavin were observed as indicated in figure 1. The dog was sacrificed after 18 weeks on experiment.

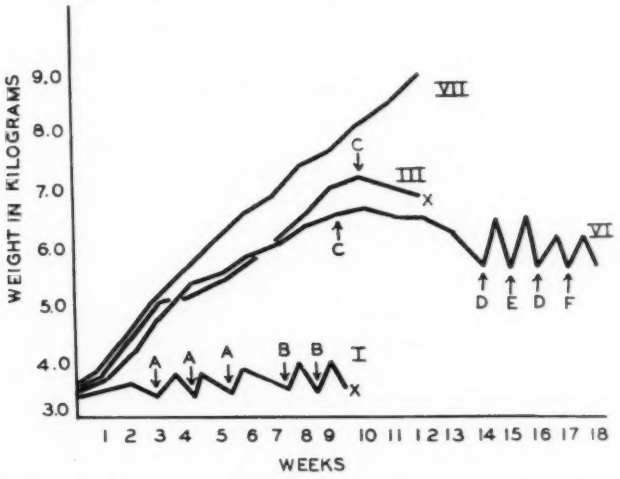

Fig. 1. Growth of dogs on riboflavin deficient ration plus varying amounts of riboflavin. Dog I received only the basal ration plus riboflavin supplementation at the points indicated. Dogs III, VI and VII received the basal ration plus a daily supplementation of 100, 200 and 400 micrograms of riboflavin per 100 grams of ration, respectively. A, 300 micrograms of riboflavin per kilogram of body weight, orally. B, 300 micrograms of riboflavin per kilogram of body weight, by subcutaneous injection. C, daily riboflavin supplementation was discontinued. D, 100 micrograms of riboflavin per kilogram of body weight, orally. E, 200 micrograms of riboflavin per kilogram of body weight, orally. F, 100 micrograms of riboflavin furnished by natural concentrate[4] per kilogram of body weight, orally.

The growth rate of dog VII which received 400 micrograms of riboflavin per 100 grams of ration is given in figure 1. A good rate of growth was observed and the dog remained healthy during the course of the experiment. The animal was sacrificed at the completion of the experiment.

Blood riboflavin determinations. A total of 6 blood samples was taken from dogs I and II while they were in acute stages of the deficiency. The riboflavin values of the 6 blood samples ranged from 0.30 to 0.38 microgram per ml. of whole blood with an average value of 0.34 microgram per ml.

[4] This sample (Solvamin) was kindly supplied by Commercial Solvents Corporation, Terre Haute, Indiana.

A blood sample taken from dog I while she was in a comatose condition had a riboflavin content of 0.30 microgram per ml. Blood samples were also taken from dog VII and from three dogs in the stock colony. These values ranged from 0.46 to 0.49 microgram per ml. of whole blood with an average value of 0.48 microgram of riboflavin per ml.

Urinary riboflavin studies ("saturation" tests).[5] The "saturation" tests were performed in the following manner on dogs I, II, IV and V while they were in the severe stages of the deficiency. The daily urinary excretion of riboflavin was first determined over a period of 3 to 4 days and the riboflavin was then administered either orally or by subcutaneous injection in doses ranging from 100 to 400 micrograms per kilogram of body weight. The riboflavin content of the following 24 hour urine specimens was determined. In most cases, the urinary riboflavin excretion reached the normal basal level within 24 hours after the administration of the test dose. "Saturation" tests were performed on two normal dogs in the same manner. The results of these studies are given in table 1. In the case of dog II identical results were obtained when synthetic riboflavin and a natural concentrate of riboflavin were given orally.

Blood chemistry studies. Blood glucose, non-protein nitrogen, urea nitrogen, uric acid and hemoglobin determinations were carried out on all of the dogs during varying stages of the deficiency. A total of 5 blood samples was taken from each dog for these studies. The hemoglobin values ranged from 9 to 12 mgm. per cent and did not appear to be a function of the degree of riboflavin deficiency. Values for blood urea nitrogen, uric acid and non-protein nitrogen remained within the normal range for the dog. Very low blood glucose values (between 20–30 mgm. per cent) were found in many cases when the dogs were in the acute stages of the deficiency. However, these results were not consistent and their correlation with the degree of riboflavin deficiency remains questionable.

Discussion. It is apparent that in our experiments the rate of growth was not a satisfactory indication of the riboflavin requirement of the growing dog. A somewhat better indication was given by the time required for the production of acute riboflavin deficiency symptoms after the removal of riboflavin from the diet. Thus, 2 dogs receiving 100 micrograms of riboflavin per 100 grams of ration began to lose weight immediately after the elimination of the daily riboflavin supplement. One of the dogs died within 2 weeks, while the other was brought to a stage of collapse within 2 weeks and was resuscitated by the administration of riboflavin. Dog VI, receiving 200 micrograms of riboflavin per 100 grams of ration, maintained weight for 3 weeks following the elimination of the daily riboflavin supplement and although losing weight was in good outward condition 2 weeks later. The data indicate that storage of riboflavin

[5] We are indebted to Mr. Richard L. Potter for assistance in these studies.

is minimal at 100 micrograms per 100 grams of ration and that a larger storage is attained at a level of 200 micrograms per 100 grams. However, one dog receiving 200 micrograms per 100 grams of ration succumbed after 7 weeks on experiment. We may then conclude that 200 micrograms per 100 grams is a minimal level and that 400 micrograms of riboflavin per

TABLE 1

The daily urinary riboflavin excretion and the retention of riboflavin in normal and in riboflavin-deficient dogs

DOG	DAILY URINARY RIBOFLAVIN EXCRETION, PER KILOGRAM OF BODY WEIGHT*	"SATURATION" TESTS		
		Dose of riboflavin, per kilogram of body weight	Mode of administration	Percentage of test dose excreted
	micrograms	*micrograms*		
I (a)	4.0	150	Oral	15
(b)		300	Subcutaneous injection	9
(c)		300	Subcutaneous injection	25
(d)		300	Subcutaneous injection	5
II (a)	6.1	400	Oral	2
(b)		400	Oral†	4
(c)		400	Oral	4
IV (a)	2.6	100	Oral	2
(b)		300	Subcutaneous injection	2
V (a)	6.0	300	Subcutaneous injection	4
(b)		300	Subcutaneous injection	11
Normal (a)	100	300	Oral	44
(b)		300	Subcutaneous injection	100
(c)		300	Oral	32
(d)		300	Oral	22
(e)		300	Oral	33
Normal (a)	98	300	Subcutaneous injection	100
(b)		300	Oral	36

* These values represent the average daily riboflavin excretion over a period of 5-10 days.

† The riboflavin was furnished by a natural concentrate. In all other cases, synthetic riboflavin was employed.

100 grams of ration furnished an adequate amount of riboflavin for the growing dog. On the basis of body weight, this level would be equivalent to 100 to 200 micrograms of riboflavin per kilogram of body weight. These values are higher than those quoted by Street and Cowgill for adult dogs receiving a restricted caloric intake (6).

The daily urinary excretion of riboflavin is markedly reduced in dogs suffering from a riboflavin deficiency as compared to that of normal dogs. These findings are in agreement with those of Fraser et al. (7) and lend support to their conclusion that the nutritional status of animals respecting riboflavin can be followed by the determination of this substance in the urine. Similar results in the human have been reported by a number of investigators (4, 8, 9, 10, 11). The results of the "saturation" tests indicate that the retention of the test dose of riboflavin is considerably greater in the riboflavin-deficient dogs than in the normal dogs. Such tests may, therefore, be safely applied as a measure of the degree of saturation of the tissues with riboflavin. It is interesting to note that Axelrod and co-workers (8) could find no such correlation between the daily urinary excretion of riboflavin and the degree of retention of administered riboflavin in human subjects with multiple vitamin deficiencies. Since the dogs employed in the present study were suffering from an uncomplicated riboflavin deficiency, it is apparent that coexisting vitamin deficiencies may play an important rôle in determining the degree of retention of riboflavin.

CONCLUSIONS

1. In a study of the riboflavin requirement of the growing dog it was found that 200 micrograms of riboflavin per 100 grams of ration was a minimal level, while 400 micrograms of riboflavin per 100 grams of ration satisfied the requirement of the growing dog for riboflavin.

2. Blood urea nitrogen, non-protein nitrogen, uric acid and hemoglobin were not affected in a riboflavin deficiency. Low blood glucose values were occasionally found.

3. A decrease of 27 per cent in the blood riboflavin values was found in acute stages of riboflavin deficiency.

4. The average, daily urinary excretion of riboflavin was markedly reduced in dogs with riboflavin deficiency. Concomitantly, the ability of the deficient dogs to retain a given test dose of riboflavin was greatly increased. "Saturation" tests may, therefore, be employed in the assessment of the degree of riboflavin deficiency in the dog.

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THE RATE OF EXCRETION OF HEPARIN IN THE URINE FOLLOWING ITS INTRAVENOUS INJECTION IN THE ANESTHETIZED DOG

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It has been repeatedly observed (1, 2, 3) that the coagulation time of the blood returns to normal within a few hours after the single intravenous injection of heparin. The brief duration of this anticoagulant action suggests that the injected heparin may be inactivated, metabolized and excreted by the body. There has been some controversy in the literature as to whether heparin is excreted in the urine following its intravenous injection. Howell and MacDonald (4) and Wilander (5) reported that heparin is excreted by the kidney. Jaques (3), on the contrary, recently reported that heparin does not appear in the urine. One of us (6) has obtained a positive qualitative test for heparin on the urine of mice following the subcutaneous injection of heparin, and on the urine of dogs following the intravenous injection of heparin.

This work was done to determine the rate and total amount of heparin excreted in the urine of dogs following its intravenous injection.

METHOD. Five fasted dogs under sodium pentobarbital anesthesia (30 mgm. per kilogram of body weight) were used in these experiments. Both ureters were cannulated and the urine was collected at 10 minute intervals over a period of about 2 hours. A mild diuresis was maintained by the slow intravenous drip administration of about 250 cc. of physiological sodium chloride solution over a period of 2 hours. Each dog received an intravenous injection of 200 units of heparin per kilogram of body weight. The heparin (110 units per milligram) was obtained from the Connaught Laboratories of the University of Toronto. Quantitative determinations of the heparin excreted in each of the 10 minute samples of urine were done by the toluidine blue method. One-half cubic centimeter of toluidine blue in distilled water (1:5000) is added to 0.5 cc. of the filtrate of urine diluted with an equal volume of physiological saline. If heparin is present a purple color results. A precipitate forms gradually,

[1] Aided by a grant from the Dazian Foundation for Medical Research.

and after 30 minutes the purple color is compared with a series of standard solutions containing from 1 to 6 units of heparin in the same amount of normal urine. If the sample of urine contains more than 6 units of heparin per 0.25 cc. it is necessary to dilute it again so that the purple color will fall within the range of the control solutions. According to Lison (7) the purple color obtained with toluidine blue is specific only for sulfuric acid esters of high molecular weight. Jorpes and Bergstroem (8) considered heparin to be a mucoitin polysulfuric ester and Jorpes (9) tested this metachromatic reaction with toluidine blue on heparin solutions. He found (10) that the color with heparin is about one hundred times more intense than it is with chondroitin sulfuric acid. We have confirmed his observations and moreover we have found that the reaction with the heparin of the Connaught Laboratories (110 units per milligram) was about 1100 times more intense than with chondroitin (Wilson & Co.) (6).

A qualitative test for the presence of heparin in the urine samples was also done on each dog by the addition of normal dog's blood to urine, diluted with an equal volume of physiological saline, and the coagulation time determined by the Howell method. Five cubic centimeters of blood were added to 0.6 cc. of diluted urine of dogs 1, 2 and 3, and 2.0 cc. of blood added to 0.5 cc. of diluted urine of dogs 4 and 5. This was compared to the coagulation time of control samples of blood to which an equal volume of diluted normal urine of the corresponding dog had been added.

The coagulation time was done upon the blood of dog 5 before the injection of heparin and at 20 minute intervals thereafter.

Results. Our results show (tables 1 and 2) that heparin is excreted in the urine following its intravenous injection in doses of 200 units per kilogram of body weight. This was evidenced by the purple metachromatic color change and by the prolongation of the coagulation time of normal blood to which urine of the heparinized dog was added. In each case the first 5 minute sample of urine collected after the injection was found not to contain heparin. Heparin, however, did appear in the 10 minute sample of urine in 3 of the 5 dogs. It is very likely that the heparin was excreted very shortly after its injection but because our ureteral catheters had a volume of about 5 cc. we did not detect its presence earlier than the 10 minute sample. Quantitative determinations on each sample of urine (table 1) show that the greatest amount of heparin was excreted in the urine between 20 and 50 minutes after its intravenous injection. After 90 minutes the amount of heparin excreted was greatly decreased. It was still present after 160 minutes in the urine of dog 5 and after 227 minutes in the urine of dog 3. Table 2 shows that from 9.9 to 35.6 per cent of the injected heparin was excreted in the urine of five dogs within 110 minutes after the injection.

Discussion. Our results show that the injected heparin was excreted in the urine and that its most rapid elimination occurred within one hour following the injection. From 9.9 to 35.6 per cent of the injected heparin was excreted in the urine within 110 minutes. The data of Wilander show

TABLE 1

The rate of excretion of heparin in the urine following its intravenous injection (200 units per kgm.) in the dog under sodium pentobarbital anesthesia

DOG		TIME IN MINUTES										
		10	20	30	40	50	60	70	80	90	100	110
1	Urine, cc.	4.0	6.6	4.5	5.8	6.4	9.5	11.0	14.0	10.0	12.5	
	Heparin, units	8	79	108	81	64	10	11	7	5	6	
2	Urine, cc.	1.6	1.4	1.6	4.2	2.6	2.6	3.2	2.2	2.4		8.0
	Heparin, units	6	11	16	67	21	10	13	18	29		64
3	Urine, cc.	1.6	2.4	1.8	1.0	1.5	2.1	2.1	2.2	1.6		2.4
	Heparin, units	0	19	22	16	18	17	17	18	13		29
4	Urine, cc.	7.6	13.6	15.4	16.1	15.9	12.1	9.1	7.5	5.3		10
	Heparin, units	243	272	370	193	127	97	73	6.0	21		40
5	Urine, cc.	1.8	2.4	2.4	2.2	2.0	2.0	2.3	2.2	2.0	2.0	1.4
	Heparin, units	0	38	58	53	48	48	55	53	32	48	11
	Coag. t.*		100		80		50		35		20	

* Normal coagulation time before the injection of heparin was 5.5 minutes.

TABLE 2

The total amount of heparin excreted in the urine following its intravenous injection in the anesthetized dog

DOG	HEPARIN			TOTAL VOLUME OF URINE	BLOOD COAGULATION TIME WITH URINE OF DOG	
	Injected	Recovered	Per cent excreted in urine		Normal	Heparinized
	units	*units*		*cc.*	*minutes*	*minutes*
1	2,200	379	17.2	84.3	4.5	10
2	2,300	255	11.1	29.8	7	23
3	1,700	169	9.9	18.7	4	10
4	4,200	1,496	35.6	112.6	7	23
5	2,400	444	18.5	22.7	10	56

that from 6 to 40 per cent of the injected heparin was excreted in the urine of narcotized rabbits within 2 hours. Jaques concluded that heparin was not excreted in the urine of the dog because he did not find any significant increase in the coagulation time of normal blood with extracts of

daily samples of urine of a dog which had been injected with 4500 units of heparin. It is possible, however, that a greater portion of the small quantity of heparin excreted in such a large volume of urine might have been lost in the process of concentration, precipitation and washing. It might also be possible that the heparin excreted in the urine following its intravenous injection is altered slightly so that it is not precipitated out by the usual methods but at the same time it can prolong the coagulation time of blood and also give a positive purple color with toluidine blue. From a chemical analysis of heparin recovered from the urine of heparinized rabbits, Wilander suggests that heparin is not significantly changed in the process of excretion. He found, however, that the recovered heparin was less effective biologically than the original heparin.

Our results verify those of Wilander and of Howell and MacDonald in that heparin is excreted by the kidney. The early excretion of the injected heparin is associated with the rate of disappearance of the anticoagulant action of heparin upon the circulating blood.

CONCLUSIONS

1. Heparin is excreted in the urine of dogs anesthetized with sodium pentobarbital following the intravenous injection of heparin in doses of 200 units per kilogram of body weight.

2. The presence of heparin in the urine was indicated by the metachromatic reaction with toluidine blue and a prolonged coagulation time by the addition of normal blood to the urine.

3. The amount of heparin excreted by the kidneys was determined by the toluidine blue method.

4. The excretion of heparin reached its highest level within 20 to 50 minutes after its injection.

5. A total of 9.9 to 35.6 per cent of the injected heparin was excreted in the urine within 110 minutes.

6. The disappearance of heparin from the urine is associated with a return toward normal of the coagulation time of the blood of the heparinized dog.

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LOWERED SERUM LIPID LEVELS IN THE ECK FISTULA DOG

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A wealth of evidence has been accumulated to show the important part played by the liver in fat metabolism, and no recent reviewer has failed to emphasize the predominant rôle played by this organ. It is known that processes of phosphorylation and desaturation which fatty acids may undergo prior to utilization take place largely in the liver (1, 2, 3), and it is reasonable to assume that the demonstrated saturation, elongation and conversion (4, 5) may also take place there. That thiamin and perhaps other vitamins are necessary for the conversion of carbohydrates to fatty acids has been shown, and this process probably occurs in the liver (6). Frazer (7) has suggested that certain types of liver damage interfere with changes in the molecular structure of the fatty acids normally brought about in this organ and therefore lead to an accumulation of unchanged fatty acids and to fatty livers. This explanation may account for the decreased plasma level and iodine number of fatty acids after carbon tetrachloride poisoning (8). We are reporting here certain studies that antedated and stimulated the investigations on carbon tetrachloride poisoning in rats (2); it is significant that similar effects on blood fatty acids are produced by dissimilar methods of depressing liver function.

METHODS. The total fatty acid and cholesterol content of fasting serum was determined on a series of Eck fistula animals, using the analytical methods described by Bloor (9, 10). It was noted at once that there was a striking correlation between the clinical state of these animals and the serum lipid level. To establish this observation single determinations were made on a series of fasting Eck fistula dogs over a period of a year, correlating the nutritional state with the serum fatty acid and cholesterol values.

As a further means of following the effect of a diminution of liver function on fat metabolism, a series was compiled in which the fat tolerance curves of normal and Eck fistula dogs were computed according to the method of Rony and Ching (11). After fasting for a period ranging from 18 hours to 3 days a control blood sample was drawn. Linseed oil, in doses of 4 and 10 ml. per kilo, was then given by stomach tube and blood

samples were drawn at 1, 3, and 6 hours after the fat meal. The total serum fatty acids were determined by the method of Man and Gildea (12), the results being expressed in milligrams per 100 ml. of serum.

Fecal fat was determined for 24 hour periods in a group of Eck fistula and normal dogs by means of the method described by Saxon (13).

Results. Table 1 shows clearly the correspondence of the blood lipid level with the general condition of the Eck fistula dog. When the animals were in good condition, as immediately after the operation, the fatty acid and cholesterol values approached the normal range. As the animals lost weight the level of the lipid fell, and when the body weight had reached its lowest levels the serum lipid levels were found to be roughly half those of normal animals. The weight loss was judged to be due chiefly to loss of body fat. For comparison, an average of the total serum fatty acids and cholesterol of 9 normal dogs determined at the time and by the same methods is included in table 1.

Table 2 shows that not only did the serum lipid values fall corresponding to the development of liver insufficiency, but the ability of the animals to respond to a fat meal with a rise in blood fat was also impaired. Increasing the amount of fat ingested did not improve this deficiency. Further, we were able in two animals to test the alimentary lipemia during the development of liver insufficiency after the establishment of the Eck fistula. Table 3 shows that there is a stage after the initial fall in fasting serum fatty acids when the animal can respond with a normal rise in blood fat to a fat meal. This ability was lost in the 2 dogs so studied some time between the third and sixth weeks after operation.

Since Whipple and Hooper (14) have shown that in Eck fistula dogs the output of bile acids is decreased to one-third or one-fourth the normal value, and since bile acids are generally believed necessary for fat absorption, the possibility of a deficiency in these substances being responsible for the failure of a rise in the fat tolerance curve was tested. In 4 trials the bile acids in the amount of 0.6 gram per kilo were suspended in the fat when it was given, but no lipemia resulted (table 2). As a further test of absorption the fecal fat of Eck fistula dogs was determined for 24 hour periods before and after the fat meal. Table 4 shows that no greater increase of fecal fat occurred following the administration of fat to Eck fistula dogs than was observed in normal animals.

Discussion. The usual post-operative course of the Eck fistula dog is characterized by a loss of weight commonly amounting to one-third of the original body weight, a normal or increased appetite, periods of "intoxication" appearing spontaneously or after the ingestion of large quantities of meat, an increased urinary output and water consumption (15), and death within about two years. In the experience of one of us (L. A. C., Jr.), who has prepared upwards of 80 such animals, a few dogs may remain

TABLE 1

Correlation of nutritional condition of Eck fistula dogs with fasting serum lipid levels

DOG	DATE OF OPERATION	DATE OF OBSERVATION	WEIGHT	CONDITION	SERUM LIPIDS	
					Total fatty acids	Total cholesterol
			kgm.		*mgm. per cent*	*mgm. per cent*
E 1	10/ 1/32	10/ 9/33	15.0	Good	295	175
		10/21/33	15.4	Good	306	170
		10/25/33	15.0	Good	298	213
		11/18/33	15.0	Good	285	243
		12/19/33	13.5	Fair	222	163
		1/28/34	13.8	Fair	249	119
		3/ 1/34	12.5	Fair	245	192
		3/18/34	12.0	Poor	194	133
E 2	6/20/33	6/29/33	20.0	Fair	215	102
		12/14/33	20.2	Fair	257	87
		1/ 2/34	18.5	Fair	203	130
		1/16/34	18.0	Poor	194	104
		1/23/35	17.5	Fair	203	86
		3/ 1/35	16.5	Poor	194	94
		3/21/35	15.5	Poor	185	92
E 3	6/21/33	9/19/33	15.0	Fair	231	125
		10/ 2/33	14.0	Poor	179	94
		12/14/33	13.5	Poor	222	87
		12/20/33	11.5	Poor	175	100
		1/12/34	10.0	Poor	157	83
		1/26/34	12.9	Fair	240	90
		2/ 7/34	12.2	Fair	268	100
		3/ 1/34	12.3	Fair	240	112
E 4	8/10/33	9/10/33	6.0	Fair	225	108
		11/18/33	5.5	Fair	194	108
		12/ 2/33	5.5	Fair	203	130
		12/12/33	5.4	Fair	222	103
		2/11/34	6.5	Fair	200	102
E 5	11/27/33	12/ 4/33	17.5	Good	277	197
		12/ 9/33	15.0	Fair	231	89
		2/ 4/35	14.5	Fair	249	121
		2/11/35	14.0	Fair	222	125
E 6	11/28/33	12/ 4/33	18.0	Good	333	144
		12/ 9/33	17.0	Fair	240	128
		12/14/34	15.5	Poor	222	83
E 7	11/31/33	12/ 9/33	14.5	Good	243	114
		12/14/33	14.0	Fair	212	95
		1/28/34	13.4	Fair	303	113
		3/ 1/34	11.4	Fair	249	130
Average of 9 normal dogs				Excellent	383	211

TABLE 2

Fat tolerance curves

ECK FISTULA						NORMAL					
Dog	Dose linseed oil	Serum total fatty acids (mgm. per cent) Hours after fat meal				Dog	Dose linseed oil	Serum total fatty acids (mgm. per cent) Hours after fat meal			
		0	1	3	6			0	1	3	6
	ml./kgm.						*ml./kgm.*				
E 1	4	269	239	232	224	1	4	560	547	662	575
E 1	4	175	161	170	167	2	4	325	395	444	495
E 1	4	110	113	126	151	3	4	452	490	597	570
E 2	4	170	167	175	172	4	4	385	371	452	487
E 2	4	167	178	175	172	5	4	414	423	468	495
E 3	4	253	278	269	264	6	4	503		498	541
E 4	4	213	210	213	213	7	4	325	307	350	366
E 1	10	266	229	213	188	8	4	524	530	731	597
E 2	10	307	283	286	264	9	4	431	444	471	519
E 4	10	305	307	302	299	10	4	336	379	401	379
E 1	4*	153	167	164	154	11	4	371	342	498	320
E 1	4*	156	164	164	162	12	4	449	689	659	605
E 2	4*	283	264	242	283						
E 2	4*	205	218	226	232						

* Plus 0.6 gram bile acids.

TABLE 3

Loss of alimentary hyperlipemia after establishment of Eck fistula

DOG	DATE OPERATED	DATE	NUTRITIONAL CONDITION	FAT TOLERANCE CURVES; TOTAL FATTY ACIDS (MGM. PER CENT) Hours after fat meal			
				0	1	3	6
E 1	10/22/34	10/18/34	Excellent	336	379	401	379
		11/13/34	Good	183	202	366	256
		12/ 1/34	Fair	170	167	175	172
		12/ 8/34	Fair	167	178	175	172
E 2	11/16/34	11/10/34	Excellent	452	490	597	570
		11/30/34	Good	199	286	363	299
		1/ 6/35	Fair	213	210	213	213

TABLE 4

Fecal fat excretion (grams per 24 hours)

ECK FISTULA			NORMAL		
Dog	Fasting	After fat meal	Dog	Fasting	After fat meal
E 1	11.9	11.0	1	3.0	9.7
E 2	1.1	3.3	2	4.8	6.2
E 3	3.6	4.8	3	2.2	5.2

outwardly normal and others may lose weight with great rapidity and die within a few months, but the above statements describe the usual sequence of events.

If the animal is sacrificed or succumbs spontaneously after marked weight loss has occurred, autopsy reveals the decrease in liver size that has been frequently reported, and also a striking loss of fat from the depots that appears to be more marked than might be expected as a result of simple inanition. Subcutaneous, omental, perirenal and other fat deposits disappear. The skin becomes loose and its loss of turgor gives one the impression that the animal is dehydrated, although Crandall and Anderson (16) have shown that dehydration is not present. The change in skin turgor can only be attributed to loss of subcutaneous fat. Quite similar changes may be observed in patients with liver disease, especially cirrhosis, in some of whom the loss of fat is striking while in others it does not seem to occur; this may well depend on the extent to which liver function is suppressed by the disease. As in the Eck fistula dog, loss of fat in the cirrhotic patient may occur while the food consumption is normal or above.

The loss of body fat that occurs in the presence of a normal caloric intake and without increased fat loss in the feces must be attributed to a decreased formation of fat by the body. The low blood levels demonstrated in our experiments support this view. The fact that an evidently decreased rate of fat formation and low blood fat level occur after liver function has been diminished by short circuiting the portal blood around the liver supports the view that new fat formation from non-fat precursors (liponeogenesis) may be exclusively or primarily a function of the liver. It can not be regarded as conclusive evidence for hepatic liponeogenesis since it is possible that suppression of liver function might depress fat formation by other tissues.

The failure of the Eck fistula dog to exhibit a lipemia within 6 hours after a fat meal can not, in view of our data on fecal fat loss, be attributed to failure of absorption. Two possibilities remain. It may be that when the fat depots are exhausted and the blood fat level low, fats are removed from the blood as rapidly as they are absorbed. It is equally possible that the decreased bile salt secretion of the Eck fistula dog does not permit fat absorption at a rate that will increase the blood fat level, but is sufficient for complete absorption at a slower than normal rate.

SUMMARY

1. The fasting serum total fatty acids and cholesterol of the Eck fistula dog are consistently lower than those of the normal dog.

2. There is a definite correlation between the functional state of the liver (as evidenced by the weight and nutritional condition of the Eck fistula animal) and the level of the serum total fatty acids and cholesterol.

3. The Eck fistula dog does not manifest the normal lipemic curve even when given twice the amount of fat that effectively increases the serum fatty acids of normal dogs.

4. The failure to produce an alimentary hyperlipemia in the Eck fistula animal is not due to inability of the animal to absorb fat as determined by fecal fat loss.

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THE RESISTANCE OF CENTRAL SYNAPTIC CONDUCTION TO ASPHYXIATION

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Temporary asphyxiation of the caudal part of the spinal cord often results in high extensor tone in the hind legs (van Harreveld and Marmont, 1939). It was observed, when these animals were sacrificed about two weeks after the initial asphyxiation, that the high extensor tone survived the disappearance of the reflexes in the anterior part of the body for a considerable time. The extensor tone, which has been shown to be a reflex tone, diminished slowly; but on several occasions still was present 10 min. after stopping the circulation. In one cat foot clonus was observed during that time. Since a slowly diminishing tone is not a sharp indicator of reflex activity, the action potentials led off from an anterior root, and elicited by stimulation of the corresponding posterior root, were used in an investigation of the survival time during asphyxiation of the cord.

METHOD. In cats, narcotized with ether, the dura was ligated at Th 12–13, severing the spinal cord at that region. One or two days later the isolated part of the spinal cord was asphyxiated for 25 to 35 min., by forcing Ringer's solution, heated to body temperature, into the ligated part of the dural cavity under a pressure higher than the blood pressure (van Harreveld and Marmont, 1939).

From 2 days to 4 weeks later the anterior and posterior roots of one of the spinal nerves (usually S 2) were prepared and placed on silver-silver chloride electrodes. Cord and roots were covered with mineral oil to prevent drying. The animal was then decerebrated and placed in a shielding metal box which was kept at body temperature. The ether narcosis given during these operations was then discontinued. The action potentials in the anterior root were recorded with a cathode ray oscillograph synchronized with a thyratron stimulator producing double shocks with a variable interval. In part of the experiments a set-up was used with which single sweeps were recorded at 5 sec. intervals during the survival of reflex activity.[1] In other experiments another set-up was used with

[1] Dr. R. Lorente de Nó brought this instrument to the California Institute of Technology during his visit in 1940, and was kind enough to allow me to use it during his stay.

which the posterior root was stimulated at intervals of 10 to 15 sec. with 30 double shocks per second for a short period.

Two ways of stopping the oxygen supply of the spinal cord were employed. In most experiments this was brought about by cutting the abdominal aorta. This stops the circulation almost immediately, reducing the blood pressure to zero. In some experiments the animal was subjected to artificial respiration with nitrogen.

The period of survival. The survival time is the interval between cutting the abdominal aorta or feeding nitrogen into the apparatus for artificial respiration and the moment at which, with maximal amplification of the oscillograph, the last action potential is seen.

TABLE 1

Survival of reflex action potentials of spinal animals

PERIOD AFTER SEVERING OF THE SPINAL CORD					
2 days		2-3 weeks		2 days	
After transection of the aorta				After breathing N 2	
Number	Survival	Number	Survival	Number	Survival
1*	2 min. 50 sec.	7*	3 min. 40 sec.	11	3 min. 20 sec.
2*	4 min. 25 sec.	8*	2 min. 25 sec.	12	3 min. 20 sec.
3*	2 min. 45 sec.	9	4 min. 35 sec.	13	3 min.
4	2 min. 50 sec.	10	3 min. 40 sec.	14	4 min. 15 sec.
5	3 min. 15 sec.				
6*	2 min. 55 sec.				

The asterisks given to some of these experiments indicate that the preparation has been examined with double shocks at long intervals; in the other experiments repeated double shocks (30/sec.) have been used.

Survival period in control animals. Table 1 shows the survival periods in spinal control animals. In one group of cats the survival time after transection of the aorta was determined 2 days after severing the cord; in a second group the survival period was determined after 2 to 3 weeks. In a third group anoxia was caused by administering nitrogen. The survival time in the first group is perhaps a little shorter than in the other groups. However, neither the manner of producing asphyxia nor the period after the transection of the spinal cord had a significant influence on the survival time. The average survival period was 3 min. 22 sec., the range being from 2 min. 25 sec. to 4 min. 35 sec.

Survival period in cats asphyxiated 2 to 4 weeks before, for 35 min. Cats were subjected to 35 min. of spinal asphyxia and 2 to 4 weeks later they were again asphyxiated. The survival times of the action potentials during this second asphyxia and other data are shown in table 2. All these

animals showed some extensor tone in the hind legs and in the tail; sometimes this tone was high. With one exception the flexion reflex was absent. Reflexes elicited by pinching the tail were absent or weak. The period of survival in these animals after cutting the aorta was considerably longer

TABLE 2

Survival of reflex action potentials in cats in which the spinal cord had been asphyxiated before

NUMBER	PERIOD OF ASPHYXIA	PERIOD OF RECOVERY	EXTENSOR TONE	FLEXION REFLEX	TAIL REFLEX	PERIOD OF SURVIVAL	DAMAGE
			After cutting of abdominal aorta				
	min.	*days*					
15*	35	16	+	−	(+)	8 min. 30 sec.	+
16*	35	27	+	+	+	8 min. 30 sec.	+
17*	35	14	+	−	−	13 min. 30 sec.	++
18*	35	14	(+)	−	−	8 min. 10 sec.	++
19*	35	17	++	−	(+)	12 min. 35 sec.	++
20*	35	12	++	−	−	13 min. 10 sec.	+++
21*	35	14	+	−	(+)	13 min. 40 sec.	+++
22	35	13	++	−	(+)	10 min.	+++
23	35	13	++	−	−	13 min. 15 sec.	+++
24	35	28	+	−	(+)	9 min. 15 sec.	+
25	35	27	+	−	−	10 min. 40 sec.	+++
26*	30	16	(+)	++	+	4 min. 20 sec.	−
27*	30	12	++	−	−	7 min. 35 sec.	++
28*	30	14	(+)	++	(+)	5 min. 20 sec.	+
29	25	15	++	−	(+)	10 min. 15 sec.	++
30	25	16	(+)	++	++	5 min. 20 sec.	+
			After breathing nitrogen				
31	35	16	+	−	(+)	14 min. 15 sec.	
32	35	16	+	−	−	17 min. 45 sec.	
33	35	15	+	−	−	13 min. 30 sec.	++
34	35	13	++	−	−	14 min. 20 sec.	+++
35	35	17	+	−	(+)	14 min. 30 sec.	+++

The signs in the columns under "extensor tone, flexion reflex and tail reflex" have the following meaning. When these reflexes were absent, this was indicated with −, when they were moderately strong, with +, and when they were pronounced, with ++. The signs in the column under "damage" have been explained in the text. The asterisks given to some of these experiments have the same meaning as in table 1.

than in the spinal control cats. The average survival time was 11 min. 2 sec., the range being from 8 min. 10 sec. to 13 min. 40 sec.

In the five cats of this group in which anoxia was produced by feeding nitrogen into the apparatus for artificial respiration a longer survival time was found than in similar animals after severing the aorta. The average

survival period of these five cats was 14 min. 52 sec., the minimum and maximum 13 min. 30 sec. and 17 min. 45 sec.

Survival period in cats asphyxiated for 25 and 30 min. 2 weeks before. Survival time and other data of cats asphyxiated for 25 or 30 min. and asphyxiated again 2 weeks later are also given in table 2. These animals often showed a high flexion reflex and in one case a high tail reflex. The survival period was considerably shorter than in the group discussed above. In the cats showing a high flexion reflex the survival period was only a little longer than in the controls.

Survival period in cats asphyxiated 2 to 6 days before, for 35 min. In a series of 10 experiments the development of the increased survival time after 35 min. of asphyxiation was examined. In 4 cats the survival period was determined 2 days after the initial asphyxiation. It was found to be considerably shorter than in the control animals (1 min. 10 sec., 1 min. 15 sec., 1 min. 50 sec. and 4 min. 25 sec.). In three cats kept for 3 to 4 days after the initial asphyxiation the survival period was longer (3 min. 20 sec., 3 min. 50 sec., and 4 min. 00 sec.). In three cats allowed to recover for 6 days the period of survival was definitely longer than in the controls (6 min. 40 sec., 7 min. 35 sec. and 8 min. 5 sec.). This, however, is still considerably shorter than after a recovery period of 2 weeks. The increased survival time observed after a recovery period of two weeks thus develops gradually during that period. It seems that after the first two weeks no further increase of the survival time occurs; in three cats (table 2; 16, 24 and 25) which were examined 4 weeks after the initial asphyxiation no unusually long survival periods of the reflex action currents were observed.

The reflex action potentials. In all the experiments the posterior root was stimulated with double shocks. The first or conditioning shock was usually smaller than the second or test shock, which was about maximal. The interval between the two was chosen to produce maximal facilitation (usually 2 to 5 msec.).

Action potentials in control spinal animals. The results obtained in these animals were materially the same as recently described by Renshaw (1940). The response to a conditioning or test shock was usually small, and of long duration, showing several spikes (fig. 1, A, B). If preceded by a conditioning shock the response to the test shock always showed distinct facilitation (fig. 1, C). The reflex time of the facilitated response was markedly shorter than of that caused by the conditioning or test shock alone, ranging in the various experiments from 1.1 to 1.3 msec. The leading off and stimulating electrodes were usually about 2.5 cm. away from the spinal cord. It will take 0.5 to 0.6 msec. for the stimulus to travel this distance, assuming that the reflex eliciting sensory fibers have the high conduction velocity of 80 M/sec. (Renshaw, 1940), and that the efferent fibers conduct

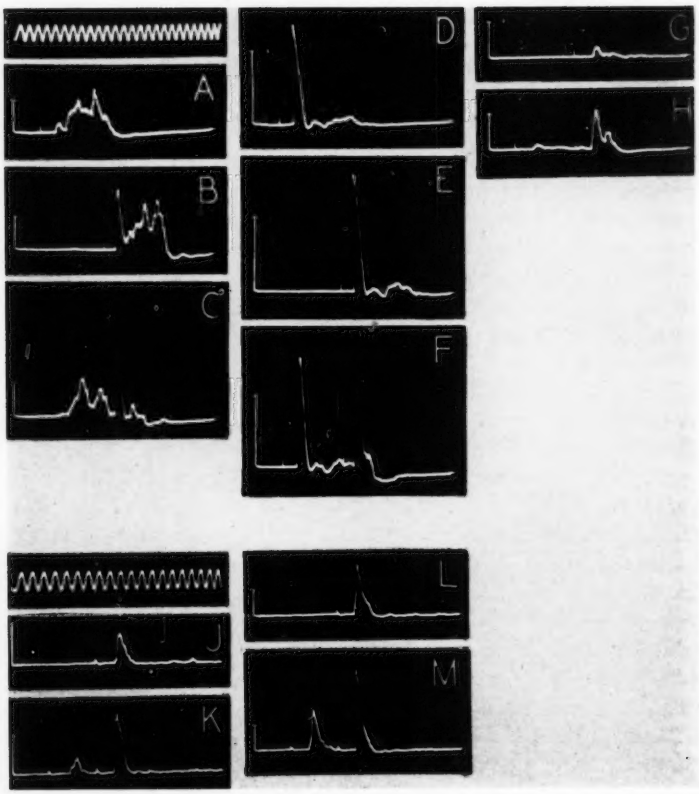


Fig. 1. A–H are reflex action potentials of a cat made spinal 2 days previously (no. 6 of table 1). A represents the response to the conditioning; B to the test shock; and C to both stimuli with an interval of 7.2 msec. D, E and F show the response to the same stimuli 1 min. 20 sec. after severing the abdominal aorta; G and H after 2 min. 35 sec. The last reflex response was seen after 2 min. 55 sec. The calibration is 0.5 mv. in A–F and 0.25 mv. in G and H. Time $\frac{1}{1000}$ sec. The reflex times from A until H are: 1.8, 1.3, 1.8 and 1.1, 1.8, 1.5, 1.8 and 1.3, 2.0, 2.0 and 1.9 msec. A marked increase of the monosynaptic spike following the conditioning shock can be seen 1 min. 20 sec. after severing the aorta (A and D). The facilitated responses to the test shock in C and F run off the screen.

J–M are reflex action potentials of a cat in which the spinal cord had been asphyxiated for 35 min. 14 days previously (no. 17 of table 2). J is the response to the test shock, K shows the responses to the conditioning and to the facilitated test shock. L and M represent similar responses 2 min. 45 sec. after severing the aorta. The stimulus interval is 4.3 msec. Calibration is 0.5 mv., time $\frac{1}{1000}$ sec. The reflex times are from J to M: 1.9, 2.0 and 1.6, 1.7, 1.7 and 1.6 msec. The reflex responses 2 min. 45 sec. after severing the aorta are materially increased. Reflex activity was observed until 13 min. 30 sec. after transection of the aorta.

at 100 M/sec. This subtracted from the reflex time gives a reduced reflex time of 0.5 to 0.8 msec. Lorente de Nó (1938) found a synaptic delay of 0.5 to 0.9 msec. for the motoneurons of the oculomotor nucleus. Renshaw (1940) found a similar value for the synaptic delay of the spinal motoneuron. Thus we will have to consider the first spike of the present reflex action potential as a monosynaptic response. It is this monosynaptic response which increases greatly by facilitation.

About 40 to 50 sec. after transection of the abdominal aorta the monosynaptic spike usually begins to grow considerably. In most cases the other spikes of the action potentials became less prominent as the monosynaptic spike grew, leading to a simplification of the action potential (fig. 1, D, E, F). In other experiments the action potentials remained complex until the end. After reaching a maximum the responses decreased gradually. Facilitation was observed until the end of all activity; when the test shock alone had failed to produce any response a distinct action potential could still be obtained when it was preceded by a conditioning stimulus. During the course of asphyxia the reflex time increased gradually to 2.5 to 3 msec.

Action potentials of cats asphyxiated 2 to 4 weeks before, for 35 min. The action potentials of these preparations are usually less complicated than those of the controls, consisting mainly of one large spike (fig. 1, J, K). In some preparations, however, more complicated responses have been observed. The conditioning shock had, as in the controls, a facilitating effect (fig. 1, K). The reduced reflex times of facilitated responses were a little longer than in the controls, ranging in most preparations from 0.7 to 1.2 msec. Nevertheless, it seems likely that in these cases the first spike also represents a monosynaptic response.

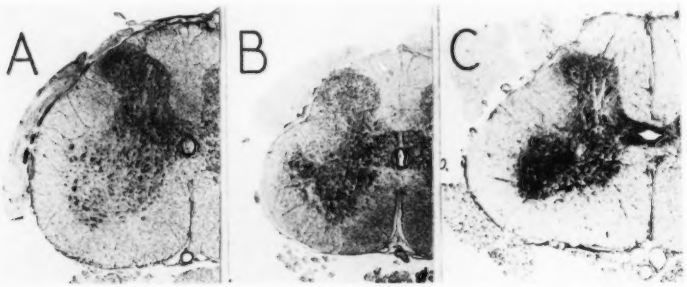
Thirty to 45 sec. after cutting the aorta the monosynaptic spike increased markedly in most of these preparations (fig. 1, L, M). In a few instances this increase was absent. After reaching a maximum the action potentials declined very slowly. The survival of these potentials was much longer than that found for the controls (table 2). A gradual increase of the reflex time during asphyxiation was observed. As in the controls a pronounced facilitating effect of the conditioning shock was present until the end. In preparations stimulated with 30 double shocks per sec. another much slower facilitation was observed shortly before reflex activity stopped altogether. The first few shocks did not cause any response, then a slowly growing response became visible, reaching its maximum after about 1 sec. These two types of facilitation are perhaps comparable with the two types mentioned by Gasser (1938). Preparations examined with 30 double shocks per second still give action potentials (after facilitation) at a time when a single double shock no longer causes a response. Thus the preparations stimulated in the former way must have a somewhat longer survival

time. The preparations stimulated with single double shocks have been marked with an asterisk in tables 1 and 2.

Histological changes in the cord. After determining the survival period, the segment of the cord used in the experiment (usually S 2, sometimes S 1) was isolated and fixed in 95 per cent alcohol. The preparation was imbedded in paraffin, sectioned (25 μ), and stained with toluidine blue.

Preparations of controls showed at this level 15 to 30 large motor cells per section. The rest of the cells in the anterior horn and in the gray commissure were mostly medium sized nerve cells. In the posterior horn the cells were very small.

In cats in which the cord had been asphyxiated for 35 min. 2 to 4 weeks before, the following changes have been observed. 1. A distinct decrease



In figure 2 three microphotographs of the spinal cord stained with toluidine blue are given. A. Of a control cat (no. 13 of table 1, survival time 3 min.). B. Of a cat asphyxiated for 35 min. 14 days before (no. 17 of table 2, survival time 13 min. 30 sec.). The middle sized ganglion cells in the anterior horn had disappeared to a great extent (++). Note the increase of nuclear material in the anterior horn. C. The cord had been asphyxiated for 35 min. 13 days before (no. 23 of table 2, survival time 13 min. 15 sec.). Few of the middle sized ganglion cells remained in the anterior horn (+++). The amount of nuclear material is exceptionally increased in this preparation.

in the number of motor cells. 2. The medium sized nerve cells in the anterior horn and gray commissure had usually decreased even more considerably. 3. The nerve cells had been replaced by large numbers of small cells (phagocytes). There were often so many of these small cells that the total amount of cell material in the gray matter was much greater than in the normal spinal cord (fig. 2, B, C). The number of cells in the white matter also increased, but to a lesser extent.

The damage as indicated in table 2 was evaluated as follows. If the number of medium sized nerve cells present in the anterior horn and in the gray commissure was about normal, this was indicated with the sign −. If there was a noticeable decrease of these cells this was indicated with +,

if they had disappeared to a great extent, with ++, and if they were practically all gone, with +++. As can be seen in table 2 there is a rough relation between the period of survival of asphyxia and the amount of damage to the cord. In all experiments in which the survival time was very long the damage was severe. In the cases in which there was only slight damage to the cord the survival time was not very long.

Discussion. A 35 min. asphyxia of the cord, although causing severe destruction in the cord, does not change the essential features of the reflex action potentials. Facilitation by a conditioning stimulus was always present; with a few exceptions the action potentials grew considerably at the beginning of a renewed asphyxiation; the reflex times were of the same order of magnitude. The only difference was that the action potentials were usually simpler, consisting mainly of one spike. This simple action potential is probably always present when a large part of the motoneurons discharges in a monosynaptic response, since a similar simplification of the action potentials has been observed in control animals when the monosynaptic response was increased by facilitation (fig. 1, B, C) or in the beginning of asphyxiation (fig. 1, A, D). The implication that in cats in which the spinal cord has been asphyxiated before, a large part of the motoneurons responds monosynaptically even without facilitation, agrees well with the observed increased reflex activity in these animals (van Harreveld and Marmont, 1939).

The great sensitivity of the central nervous system to asphyxia is well known. Sugar and Gerard (1938) reviewed the literature and determined the survival time for a number of regions of the brain, mostly using the spontaneous electrical phenomena as the indicator of activity. They found survival times ranging from 10 to 12 sec. in the cerebellum, to more than 2 min. in the tuberculum cuneatum and in the spinal tract of the trigeminus in the medulla. Heymans, Jourdan and Nowak (1934), Heymans and Bouckaert (1935), and Heymans, Bouckaert, Jourdan, Nowak and Farber (1937) determined the survival times of some reflexes of the medulla. The cornea reflex disappeared after 1 min. to 1 min. 30 sec., respiration stopped after 1 min. 30 sec. to 2 min. Cardioregulation and vasomotor reactions were paralyzed after 4 to 5 min. We found in a large number of experiments in which the caudal part of the spinal cord was brought under pressure, causing sudden circulatory arrest, that the kneejerk had an average survival time of about 45 sec., with a minimum and maximum of about 30 and 80 sec. In this paper it has been shown that when the reflex action potentials are used as the indicator, the survival time of the spinal cord is much longer (average 3 min. 22 sec.). By using the action potentials the survival time of the reflex most resistant to asphyxia is determined. This apparently is not the kneejerk.

Since a large number of nerve cells is destroyed in the asphyxiated

cord, the most obvious explanation of the increased survival time would be to assume that the oxygen in the blood and in the tissue surrounding the nerve cells would suffice for the remaining cells for a longer period than normal. There are many objections to such a point of view. 1. Though the number of ganglion cells is decreased, this is compensated and often overcompensated by the presence of large numbers of phagocytes in the damaged tissue. Even if the phagocytes have a lower metabolism than the nerve cells, it is inconceivable that the small oxygen reserve in the vessels, which according to Gerard (1937) can supply the cortex for only 10 sec., would suffice for more than 13 min. in the previously asphyxiated cord. 2. In the previously asphyxiated animals the increase of the action potentials during a renewed asphyxia begins at about the same moment as in the controls. If the increased survival period were due to a slower use of stored oxygen, the increase of the action potentials could be expected to set in later in the treated animals than in the controls. 3. In a number of experiments anoxia of the cord was caused by ample (3 l./min.) artificial respiration with nitrogen. The nitrogen removes the oxygen from the blood as it passes the lung, and since circulation proceeds for a few minutes this thoroughly venous blood will remove any oxygen which might be present in the tissues of the spinal cord. Though there was in these cases certainly no reserve of oxygen present in the cord, the survival time was even longer than when asphyxia was caused by cutting the aorta. This longer survival time may be explained by the fact that in this way carbon dioxide and other substances can be removed from the cord as long as circulation proceeds. It seems certain that in the previously asphyxiated spinal cord, synaptic conduction is considerably more resistant to oxygen lack than in the spinal cords of control animals.

It has been found that peripheral synapses are not particularly sensitive to oxygen lack. Bronk and Larrabee (1937) and Bronk (1939) found that about 30 min. after stopping the circulation conduction in the stellate ganglion begins to fail, after about 60 min. it stops altogether. Bargeton (1938) found that 10 to 15 min. after circulatory arrest transmission through the superior cervical ganglion had stopped completely. The survival time of the previously asphyxiated cord approaches that of the peripheral synapses.

If synaptic conduction is not very sensitive to oxygen lack the question arises why the reflex activity of the normal spinal cord survives asphyxia for such a short period of time. We have to assume that there is present in the spinal cord a structure highly sensitive to oxygen lack which, when asphyxiated, can depress synaptic conduction. This could be accomplished by the release of a chemical substance or by a spontaneous and continuous discharge of neurons with an inhibitory function. Since the increased survival time develops slowly in about two weeks it is probable

that this is connected with the secondary degeneration of the fibers and fiber endings of the nerve cells killed by the initial asphyxiation, rather than with the destruction of the cell bodies themselves, which occurs shortly after the initial asphyxia (van Harreveld and Marmont, 1939). Since asphyxiation of the cord causes on the one hand an increased survival time and on the other hand the destruction of the structures responsible for reflex inhibition (van Harreveld, 1939) and of structures normally depressing the reflex activity of the cord (van Harreveld and Marmont, 1939; van Harreveld, 1940) it seems quite possible that the destruction of those structures is the cause of the increased survival time.

SUMMARY

1. The survival time of reflex action potentials of the spinal cord after transection of the abdominal aorta was determined in spinal animals. The average survival time was 3 min. 22 sec., ranging from 2 min. 25 sec. to 4 min. 35 sec.

2. The survival time was determined in the same way in cats in which the spinal cord had been asphyxiated for 35 min., 2 to 4 weeks previously. The survival times in these animals were much longer than in the controls. The average survival time was 11 min. 2 sec., ranging from 8 min. 10 sec. to 13 min. 40 sec.

3. This increased survival time was not present the first few days after the initial asphyxiation, but developed gradually in the course of two weeks.

4. The initial asphyxiation destroyed a large number of ganglion cells in the cord. It was shown that the increased survival time is not due to a slower use of oxygen stored in the vessels and in the tissues surrounding the ganglion cells by the few nerve cells left, but actually is an increased resistance of synaptic conduction to asphyxiation.

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HYPOTHALAMICO-HYPOPHYSIAL SYSTEM AND ITS RELATION TO WATER BALANCE IN THE DOG

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Several recent reports (Fisher, Ingram and Ranson, 1938; Rasmussen, 1940) have dealt with various aspects of the supraoptic hypophysial system, the hypophysis and their relation to water balance. In this communication the results of a similar investigation carried out in dogs since 1935 will be presented. Certain functional studies dealing with alterations in water balance following surgical operations on the hypothalamus and the hypophysis of some of the animals of this series have already been published (White and Heinbecker, 1937). In addition to the anatomical studies of the hypothalamico-hypophysial system this report deals with the following: *a*, the rôle of the anterior lobe in diabetes insipidus; *b*, the mechanism of the so-called normal interphase; *c*, a correlation between the degree of diabetes insipidus and the residuum of nerve cells in the supraoptic nuclei; *d*, evidence that the supraoptic and paraventricular nuclei do not secrete pitressin; *e*, the effect on fluid and food intake of infection in the region of the hypothalamus in the absence of pitressin-forming tissue.

MATERIALS AND METHODS. Dogs were used as experimental animals. They were kept in metabolism cages with high, solid sides and deeply sloping bottoms to insure satisfactory 24 hour urine collections. They were fed measured amounts of dog chow and horse meat except when for experimental purposes unlimited but measured quantities were allowed. The dogs were kept for varying periods to establish the normal daily urine output before operation, and then subjected to operative procedures designed to determine the effect on water balance of the loss of the different parts of the neuro- and adenohypophysis. Anatomical material was thus also provided to determine the effect of the loss of the various parts of the neurohypophysis on the hypothalamic nuclei.

The following are the operations carried out. The oral approach was used almost exclusively: 1, removal of the posterior lobe with or without removal of the pars distalis; 2, a low or a high section of the infundibular

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stem with or without removal of the posterior lobe and pars distalis; 3, section of the fibers to the neurohypophysis with the immediate or subsequent removal of the adenohypophysis, the infundibular stem and the infundibular process; 4, removal of the neuro- and adenohypophysis at one sitting. We would emphasize that complete removal of pitressin-forming tissue must include removal of the median eminence.

While it was not possible on every occasion to carry out with precision the particular operation planned, the number of animals utilized (150) was great enough so that in the end an adequate number of successfully operated animals of each type were available for study.

After operation the animals were again followed for periods varying from 10 days to a year and the effect of each type of operation on water exchange determined. They were then sacrificed and the nature of the lesion in the hypophysis and hypothalamus and the presence or absence of pars distalis in each instance was determined by microscopic examination. The brains were fixed in situ by irrigation through the carotid artery with formalin 1 in 10 after bleeding the animal. Early in the research the hypothalamus and sellar contents were prepared for sectioning on one block. The treatment with the 5 per cent nitric acid used for decalcifying was found to interfere with the subsequent staining of many of the nerve cells with cresyl violet. Later the sella was separated from the hypothalamus after fixation. Both parts were then prepared separately and examined microscopically in serially cut 20 micron sections. Cresyl violet was used to stain the hypothalamic tissue, hematoxylin and eosin for the sella turcica and its contents.

Nomenclature. In order to avoid confusion the system of nomenclature recently suggested by Rioch and Wislocki (1940) is employed.

Major divisions and subdivisions of the mammalian hypophysis

Major Divisions	*Subdivisions*	
Adenohypophysis		
Lobus Glandularis	1. Pars distalis (anterior lobe)	
	2. Pars tuberalis	
	3. Pars intermedia	
Neurohypophysis		posterior lobe
Lobus Nervosus (Neural lobe)	1. Infundibular process	
Infundibulum (Neural stalk)	1. Infundibular stem	Neural stalk, together with sheath of lobus glandularis, designated as hypophysial stalk
	2. Median eminence of tuber cinereum	

The only nuclei which unquestionably contribute fibers to the neurohypophysis were found to be the supraoptic and the paraventricular. The supraoptic as shown in figure 1 is divisible into a tightly packed rostral division, a small intermediate medially directed spur and a relatively

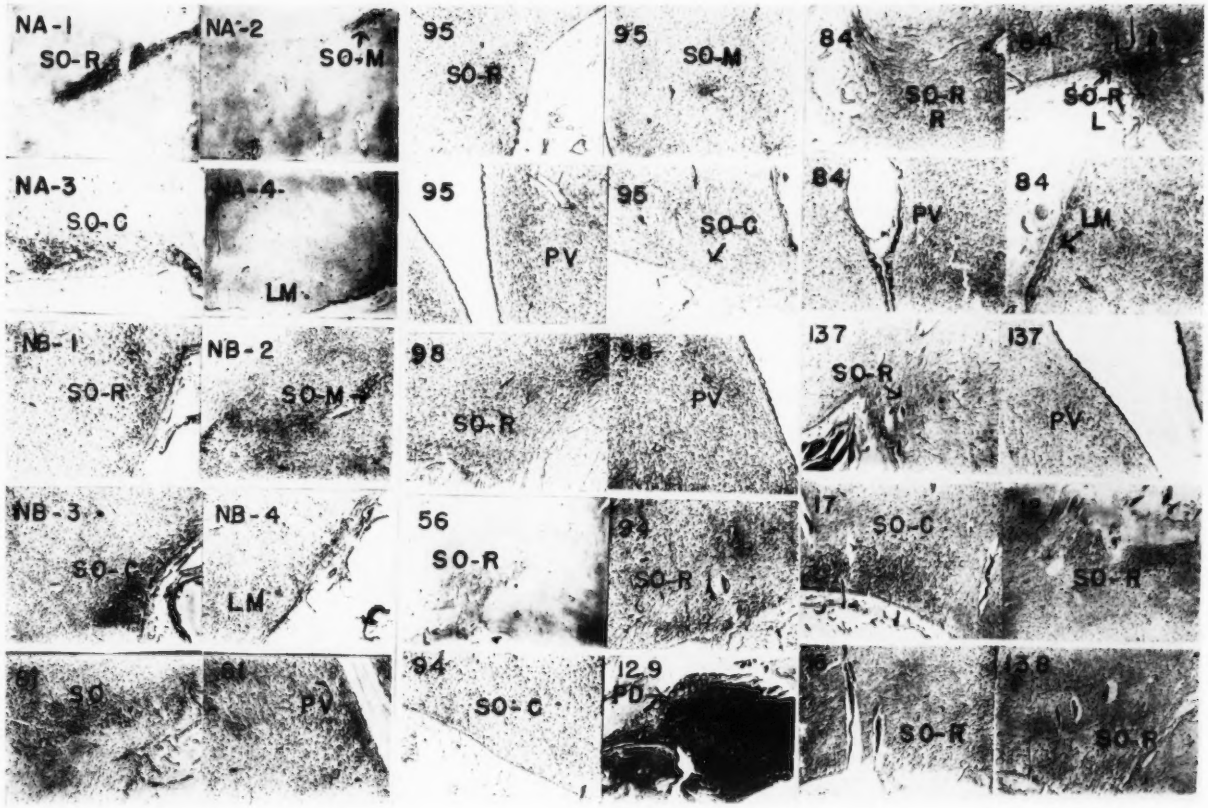
NA-1
SO-R
NA-2
SO-M
95
SO-R
95
SO-M
84
SO-R
R
84
SO-R
L
NA-3
SO-C
NA-4
LM
95
PV
95
SO-C
84
PV
84
LM
NB-1
SO-R
NB-2
SO-M
98
SO-R
98
PV
137
SO-R
137
PV
NB-3
SO-C
NB-4
LM
56
SO-R
94
SO-R
17
SO-C
SO-R
94
SO-C
12.9
PD
SO-R
SO-R

Fig. 1

diffuse caudal division commonly called the tuberal nucleus. The paraventricular nucleus is ventrally situated in its rostral division and courses dorsocaudally. The cells of the supraoptic and paraventricular nuclei are cytologically similar. They are large, with their Nissl granules peripherally located in the cytoplasm. Their nuclei often have an eccentric position. After treatment with 5 per cent nitric acid for 3 weeks these are the only hypothalamic cells which stain at all satisfactorily with cresyl violet (fig. 1).

RESULTS. To review all of our results in detail is obviously impossible. It was therefore decided to present the water exchange and anatomical findings of one typical example from each of the operative classes outlined above. An analysis of the results leads to conclusions which are applicable to the entire body of material at our disposal.

Class 1-a (dog 95). Removal of the posterior lobe and pars distalis ("simple hypophysectomy").

This procedure does not result in any permanent alteration in water

Fig. 1. Photomicrographs 150 diameters showing essentially the supraoptic and paraventricular nuclei in two normal dogs, NA and NB, and in eight operated animals the numbers of which are indicated in the upper left hand corner of each photomicrograph. The tissue of normal dog NA was treated with 5 per cent nitric acid in decalcifying the attached sella turcica. As a consequence, only the supraoptic and paraventricular nuclei stained in such sections. NA-4 is a section through the lateral mammillary nucleus, which is here unstained. *SO-R*, *SO-M*, *SO-C* indicate the rostral, intermediate and caudal divisions of the supraoptic nucleus. *PV* indicates the paraventricular nucleus, LM the lateral mammillary nucleus. NB-4 shows a lateral mammillary nucleus which stains when the tissue is not subjected to 5 per cent nitric acid. Sections from dog 81 show a few residual supraoptic cells, which accounts for a late decrease in the degree of polyuria. The caudal portion of the paraventricular nucleus is practically normal. Sections from dog 95 show a loss of from 60 to 80 per cent of the supraoptic cells; note that the intermediate division is definitely present. The loss of cells in the caudal division is as great as in the rostral division. There is a moderate loss of cells from the paraventricular nucleus especially in its rostral portion. Sections from dog 98 show a cell loss similar to those for dog 95. The supraoptic nuclei in dog 56 and dog 94 show about a 90 per cent loss of cells. The section from dog 129 shows normal appearing pars distalis removed at the second operation. Sections from dog 84 show practically a complete loss of supraoptic cells in both the right and left nuclei with considerable loss (estimated 50 per cent) of paraventricular cells, especially on one side. The lateral mammillary nucleus is normal, indicating that its preservation is compatible with a maximum diabetes insipidus (ventral aspect of hypothalamus is toward the left in this section, fig. 1, dog 84, LM). Sections from dog 137 show a complete loss of supraoptic cells with a 50 per cent loss of paraventricular cells. The section from dog 17 shows no loss of cells in the supraoptic nucleus 19 days after interruption of the fibers from the nucleus. The section from dog 16, 30 days after such an interruption of the fibers, shows a few remaining degenerated cells. The section from dog 138, 65 days after interruption of the fibers, shows a complete absence of cells, the dark spots are due to the staining of some tissue detritus.

exchange (fig. 2). If there is slight injury to the median eminence at operation there may be a temporary increase in urine output of 2 to 6 times the normal for 24 to 72 hours. At the end of this time the urine output returns to normal and remains there. If the median eminence is not affected at all there is not even a temporary increase in water exchange.

Microscopic examination of the hypothalamus reveals an estimated 70 to 80 per cent decrease in the cells of the rostral, median and caudal divi-

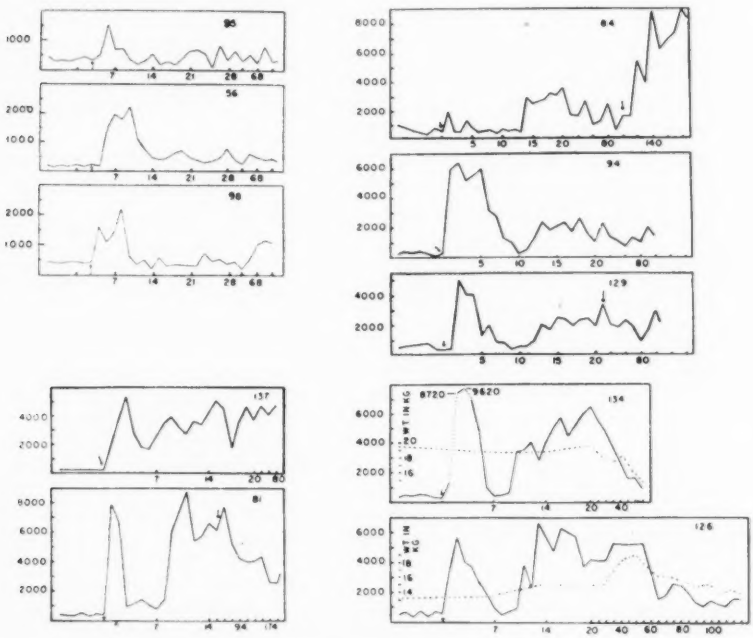

Fig. 2. Plots of urine output in cubic centimeters against time in days for dogs 95, 56, 98, 137, 81, 84, 94, 129, 134 and 126 as indicated by the number in the upper right hand corner of each chart. For description of operation see text. Arrows mark the time of an operation the nature of which is also indicated in text. Dotted lines in charts of dogs 134 and 126 indicate body weight in kilograms.

sions of the supraoptic nuclei (fig. 1). There is no obvious loss of cells in the paraventricular nuclei.

Class 1-b (dog 56). Removal of the posterior lobe without removal of the pars distalis by splitting the latter longitudinally to permit removal of the former.

The water exchange (fig. 2) and microscopic findings (fig. 1) in the hypothalamus are similar to those for class 1-a. There is always a large residuum of normally staining pars distalis.

Class 2-a (dog 98). Low section of the infundibular stem without removal of the posterior lobe and pars distalis.

This results in water exchange effects (fig. 2) and microscopic findings in the hypothalamus (fig. 1) similar to those for class 1-a. The posterior lobe is shrunken, vacuolated and quite cellular. The operation does not result in any appreciable change in the size and staining properties of the pars distalis.

Class 2-b (dog 94). High stalk section with removal of the infundibular stem, the posterior lobe and pars distalis.

This results in a temporary moderate to high polyuria which is followed in 3 to 5 days by a normal interphase (fig. 2). Following this in 3 to 7 days there usually results a permanent increase in urine output of 2 to 6 times the normal amount depending upon the degree of permanent injury to the median eminence. If there is no permanent injury to the median eminence no permanent polyuria results.

Microscopic examination of the hypothalamus (fig. 1) reveals an estimated loss of 80 to 90 per cent of the cells in each of the three divisions of the supraoptic nuclei and a variable loss of cells in the ventral part of the paraventricular nuclei.

Class 2-c (dog 129). High stalk section without removal of the infundibular stem, posterior lobe and pars distalis.

This results in similar water exchange findings (fig. 2) to those for class 2-b. Microscopic examination reveals the infundibular stem and pars distalis to be shrunken, often showing cystic degeneration and a high degree of cellularity. Subsequent removal of the pars distalis (dog 129, fig. 1) does not modify the water exchange (dog 129, fig. 2) after second arrow). The loss of cells in the supraoptic nuclei is similar to that for class 2-b.

Class 3-a (dog 84). Section of the fibers to the neurohypophysis with immediate removal of the lobus glandularis, the infundibular stem and the infundibular process.

This results in a high temporary polyuria of 4 to 6 days, a normal interphase of 3 to 5 days and then a state of maximum permanent polyuria (fig. 2). The first arrow (fig. 2, dog 84) indicates the time of an unsuccessful attempt at section of the fibers to the neurohypophysis.

Microscopic examination of the hypothalamus reveals a complete loss of cells in the three divisions of the supraoptic nuclei, a variable but marked loss of cells in the ventral portion of the paraventricular nuclei and a variable but much smaller loss of cells in the dorsocaudal portion of the nuclei (fig. 1). A unilateral interruption of the fibers results in a discernible loss of nuclear cells on the same side only. The degree of completeness of removal of the lobus glandularis was established by examination of serial sections of the entire sella and its contents.

Class 3-b (dog 81). Section of the fibers to the neurohypophysis with a

subsequent removal of the adenohypophysis, the infundibular stem and the infundibular process at a second operation.

The water exchange in this class is similar to that for class 3-a. The second operation at which the adenohypophysis is removed results in no diminution in water exchange (fig. 2).

Microscopic examination of the hypothalamus reveals similar findings to those for class 3-a (fig. 1). The absence of pars nervosa was established by examination of the serial sections of the entire sella and its contents.

Class 4 (dog 137). Removal of the neuro- and adenohypophysis at one sitting.

The operation results in an immediate and permanent high polyuria with no normal interphase (fig. 2). The microscopic findings in the hypothalamus are similar to those for class 3 (fig. 1).

Time interval for nerve cell degeneration following interruption of the axons of the supraoptic nuclear cells. Analysis of our material indicates that a beginning failure of the Nissl substance to stain with cresyl violet can be noted in the supraoptic nuclear cells at 10 to 14 days and is complete in 45 to 60 days after their axons have been interrupted. In figure 1 are shown photomicrographs of the supraoptic nucleus following an anatomical lesion shown to be adequate to interrupt all fibers to the neurohypophysis at intervals varying from 19 days (dog 12) to 30 days (dog 16) and to 65 days (dog 138). These results are in agreement with similar ones reported by Rasmussen (1940) for the rat, the dog and man.

Rôle of the anterior lobe in diabetes insipidus. It has been possible to show in 9 dogs that a permanent maximum diabetes insipidus followed complete destruction of the neurohypophysis with complete absence of the pars distalis, microscopically confirmed by serial sections. It was previously reported by us that in 8 other animals a permanent maximum diabetes insipidus was produced by complete interruption of the fibers to the neurohypophysis or complete destruction of the neurohypophysis with only 3 to 10 per cent of the pars distalis remaining. We now recognize that the polyuria in these 8 animals would not have been impaired if these residual fragments of pars distalis had been removed. In the light of our entire evidence it is felt that failure to obtain maximum permanent diabetes insipidus by complete removal of the hypophysis at one sitting is due to failure to interrupt all the fibers to the neurohypophysis or to remove it completely. The presence of more than 3 per cent of intact residual cells in the supraoptic nuclei after adequate time for degeneration is incompatible with a state of permanent maximum diabetes insipidus. Much of the confusion on this point in the literature has been due to failure to recognize that complete removal or denervation of the median eminence is a necessary condition for maximum permanent polyuria.

von Hann (1918) advanced the hypothesis that some pars distalis was

essential for diabetes insipidus. His evidence was derived from an analysis of 20 clinical cases. In 9 of these it was stated that complete destruction of the hypophysis existed without diabetes insipidus. In all cases of diabetes insipidus the posterior lobe was found destroyed but the pars distalis was in whole or in part intact. In view of our findings it is considered probable that von Hann's evidence for complete destruction of the neurohypophysis was inadequate, the median eminence probably remaining. Fisher, Ingram and Ranson (1938, loc. cit.) have supported the von Hann theory. Their acceptance of it was based on an analysis of the findings of other investigators and their own evidence of a diuretic effect from an extract of beef anterior lobe in cats with a latent tendency toward diabetes insipidus. No effect was obtained in several cats already having a good polyuria. Richter (1934) also supported the von Hann theory on the basis of results obtained in the rat. His estimates of the degree of hypothalamic and hypophysial destruction in the animals seems to have been based on gross rather than microscopic examinations. The state of the median eminence and of the supraoptic and paraventricular nuclei are not reported upon. It is our belief that in those rats which fail to develop a maximum permanent diabetes insipidus this was due to a failure to destroy all pitressin forming tissue (median eminence escaped) rather than to any difference in the amount of pars distalis remaining.

The normal interphase. Fisher, Ingram and Ranson (1938, loc. cit.) called attention to the existence of a period of normal or nearly normal fluid exchange which occurs between the transient and permanent phases of polyuria. It is usually of 3 to 7 days' duration in the dog and is followed by a sudden development of the permanent phase of polyuria (dogs 81 and 84, fig. 2). The mechanism concerned in the normal interphase has remained in doubt. In a large series of our animals it has been possible without exception to eliminate the normal interphase by removal of all the pitressin-secreting tissue (dog 137, fig. 2). We interpret this as meaning that in the operation of interrupting the hypothalamico-hypophysial tract, following which this normal interphase occurs, there is sufficient injury to the pitressin-forming tissue to prevent hormone secretion, which leads to a transient polyuria phase. After subsidence of the effects of trauma the pituicytes resume their secretory activity, which again disappears after 3 to 10 days because of the loss of trophic nerve influences necessary for their function. This final phase is permanent because the pituicytes now actually undergo degeneration. Complete removal of all pitressin-forming tissue invariably results in the immediate development of a permanent polyuria even though no pars distalis is present (dog 137, fig. 2). This shows that the normal interphase cannot be referred to any variations in activity of the pars distalis.

Correlation between the degree of diabetes insipidus and the degree of supra-

optic nerve cell degeneration. In figure 3 is plotted the relationship between the number of residual cells in the supraoptic nuclei of operated animals and the degree of resulting polyuria after interruption of the supraopticohypophysial tract or removal of the neurohypophysis. Each dot represents the findings in one animal. The number of the residual cells was estimated by counting the cells in the supraoptic nuclei in a number of sections through the three portions of the supraoptic nucleus and by measurements which permitted an estimate of the volume of the remaining nucleus. From this an estimate of the number of nuclear cells was arrived at and the result compared with the number of cells in an average normal

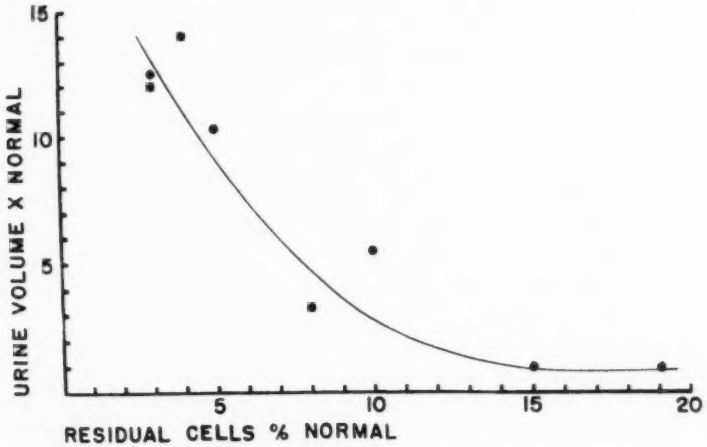

Fig. 3. Plot of the relationship between the number of residual cells in the supraoptic nuclei of eight operated animals and the degree of polyuria. Abscissa-percentage of supraoptic nuclear cells expressed as percentage of cells present when compared with the number in normal supraoptic nuclei; ordinate-urine volume expressed as the number of times normal for the particular dog.

nucleus. It is recognized that the results are necessarily approximations but it is felt that they are nevertheless similar to results which would follow from a complete count of all the remaining cells.

From the nature of the operative procedures used it follows that complete severance of the supraoptic nuclear cells from the neurohypophysis also results in a severance of whatever paraventricular cells are normally connected with it.

The question of pitressin secretion by the supraoptic and paraventricular nuclear cells. It has been suggested by Gaup and Scharrer (1935) that the cells of the supraoptic and paraventricular nuclei may secrete pitressin and that on removal of the neurohypophysis these cells may take over the

function of pitressin production. In a more recent publication Scharrer and Scharrer (1940) admit that the evidence favoring the secretory capacity of these cells is purely histological and that the nature of their secretion remains to be established. On the basis of the evidence of our investigation this secretion cannot be pitressin. It seems reasonable to assume that a normal staining reaction of a nerve cell can be regarded as evidence of its normal functional state. In our dogs where complete removal of the pituicyte-bearing tissue is carried out at one sitting, a maximum diabetes insipidus obtains from the day of operation. For the first 10 to 12 days following the operation the cells of the supraoptic and paraventricular nuclei stain normally. During this time such cells, if essential for pitressin formation, should be capable of taking over the function of the neurohypophysis and no marked polyuria should develop.

Influence of infection in and about the hypothalamus on water and food intake in the absence of neurohypophysis. In six instances the influence of infection in and about the hypothalamus was observed in dogs where all pitressin-forming tissue had been denervated or removed. Serial sections showed complete or almost complete absence of the supraoptic nuclei and the ventral rostral portions of the paraventricular nuclei. These animals first showed a typical maximum diabetes insipidus which after several weeks began gradually to diminish and in some instances returned to normal water exchange level. With this diminution in urine output there was a simultaneous diminution in water and food intake. Some of these animals eventually died, with great loss of body weight, presumably as a result of the anorexia. The picture resembles that of so-called hypophysial cachexia (Simmonds, 1914) which is usually ascribed to loss of the anterior lobe. Our results show that in the dog complete loss of the anterior lobe alone does not produce any such symptoms and leads to the inference that hypophysial cachexia may be due to an associated hypothalamic depression or lesion rather than to anterior lobe loss alone. Typical examples of the water balance and body weight curve in this group of animals (dogs 134 and 126) are given in figure 2.

While we accept the view (Richter, 1935; Fisher, Ingram and Ranson, 1938) that polyuria is primary in diabetes insipidus, any influences which interfere with the thirst and appetite of an animal will prevent the manifestations of diabetes insipidus in spite of the fact that the anatomical basis for this state exists. That operations involving injury to the hypothalamus may interfere with thirst and appetite is known to all investigators in this field. In our experience with dogs, simple hypophysectomy does not interfere appreciably with normal thirst and appetite. When more extensive injury involving the hypothalamus is produced, interference with thirst and appetite may result. The more extensive the injury, the greater appears to be the effect. In our experience removal of the entire

hypophysis can be carried out at one sitting without much interference with thirst and appetite, provided it is effected by clean dissection rather than by probing plus chemical injury. The disturbance of thirst and appetite is frequently temporary.

CONCLUSIONS

The neurohypophysis is innervated by fibers from the supraoptic and paraventricular nuclei. All the cells of the supraoptic nuclei and a high percentage of the cells of the rostro-ventral portion of the paraventricular nuclei degenerate on removal of all the neurohypophysis. The innervation of the median eminence is apparently uncrossed.

Removal of the infundibular process results in the retrograde degeneration of 70 to 80 per cent of the supraoptic nuclei. The cells of the paraventricular nuclei show little or no degeneration. Removal of the entire infundibular stem and the infundibular process results in retrograde degeneration of 80 to 85 per cent of the cells of the supraoptic nuclei.

Following interruption of the hypothalamico-hypophysial tracts, degeneration, as evidenced by paleness of staining and breaking up of the Nissl substance, begins at 10 to 14 days. For complete degeneration 45 to 60 days are required.

Maximum and permanent diabetes insipidus follows the removal or the complete denervation of the entire neurohypophysis, resulting in retrograde degeneration of the entire supraoptic nuclei and the rostral ventral portions of the paraventricular nuclei. No nucleus caudal to the paraventricular nuclei contributes fibers to the neurohypophysis. Failure to interrupt the fibers of even 15 per cent of the cells innervating the neurohypophysis will prevent the development of any permanent diabetes insipidus. Failure to interrupt 5 per cent of these fibers will result in a diabetes insipidus of only 4 to 5 times the normal urine output, while this output is increased 10 to 20 fold with complete interruption.

The normal interphase represents the time during which the denervated neurohypophysis produces pitressin.

The adenohypophysis is not necessary for the development and maintenance of a permanent and maximum state of diabetes insipidus in the dog.

Evidence is presented that infection in the region of the hypothalamus can prevent the manifestation of diabetes insipidus even in the absence of the entire neurohypophysis.

Evidence is presented that the cells of the supraoptic and paraventricular nuclei do not secrete pitressin.

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RÔLE OF THE NEOSTRIATUM[1]

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Some time ago, in a study of extrapyramidal function, it was reported (1) that stimulation of the caudate nucleus produced an "inhibition" of movements already in progress. The essential results obtained in that investigation were: 1, if an animal were struggling, stimulation of the caudate nucleus or putamen stilled this activity, and 2, if a specific movement were introduced (phasic flexion of the forepaw) by stimulation of the cortex this movement was reduced in amplitude, frequency or duration of movement and, sometimes, stopped altogether by stimulation of the caudate or putamen. In reporting the original results it was decided not to emphasize the first observation since spontaneous movement is so easily affected by a variety of circumstances including sensory stimulation. Further we were far from satisfied with the application of the word "inhibition" to a subsidence of spontaneous activity. This term has come to have specific limitations attached to it which are difficult to apply in forebrain work. In the present communication, for want of a better term, we still continue to speak of inhibition in the sense in which we used it before, that is, "as a 'melting-away' of the cortical effect" but we also apply it to a cessation of spontaneous activity, with the implication that the inhibition is not abrupt and that the movement gradually subsides over a brief interval of time. We have further employed the term when a significant reduction in the amplitude and frequency of vigorous, spontaneous movements was obtained.

METHODS. Adult cats were employed in the stimulation experiments listed below. The animals were supported longitudinally on a horizontal bar, and equipped with a wide-frame Horsley-Clarke apparatus. Two types of electrodes were employed for deep stimulation. The first of these was of the monopolar variety and consisted of a tungsten or steel wire insulated to the tip, the indifferent electrode being attached either to the frame of the stereotaxic instrument or to the rump of the animal. The second type was the bipolar concentric.

In stimulating the cortex we have employed the ordinary bipolar, silver,

[1] Financial assistance from the John and Mary R. Markle Foundation is gratefully acknowledged.

forked electrode; the monopolar, silver, ball-tip (2 mm.); the monopolar silver, spring filament (contact area *circa* 0.25 mm.) and the saline capillary plug. As stimulating sources the spring-inductorium, half-rectified sixty-cycle and variable-frequency thyraton stimulator have all been used. Generally, we have used the sixty-cycle, half-rectified stimulator for cortical work and the variable frequency apparatus for deep placements. Usually the latter was set for 60 pulses per second though a slower frequency was sometimes more effective.

Ether anesthesia delivered through an intratracheal cannula was employed during all operative procedures. During stimulation the level of the anesthesia was reduced to the first or second stages of Hewitt.

Relative activity of different portions of caudate nucleus. In studies made with the intention of investigating the possibility of somatotopical representation in the caudate it was observed that stimulation in all parts of the nucleus was not equally effective in inhibiting cortically induced movements. It was further observed that, while the caudate exerted a greater inhibiting effect upon movements elicited from the cortex of the same hemisphere, spontaneous activity was as completely inhibited on the same as opposite side of the body and indeed that no selective inhibition of spontaneous activity could be observed at all. With a view to determining 1, whether the areas of the caudate which produce inhibition of spontaneous movement are more or less coextensive with those inhibiting cortically induced activity, and 2, whether the regions producing inhibition of induced movement have some particular pattern, it was decided to construct a map of the caudate based upon its stimulation. Two hundred individual placements were made in this experiment. In only two or three instances were more than two placements made in any one animal (one in each caudate). It was possible to verify the exact position of 166 of these placements. For the sake of convenience the caudate was subdivided into six regions; the anterior, middle and posterior third, each of the head and body. Roughly the posterior boundaries of these regions correspond respectively with the six drawings (A through F) shown in the accompanying figure (which also contains the results of the putamen stimulations discussed later in the paper). The amount of caudate tissue comprised in each of these regions varies considerably. The number of placements made in each location was roughly in proportion to the amount of tissue involved and was as follows: 14, 52, 54, 22, 10 and 14 respectively. No attempt to locate placements in the tail of the caudate was made since the possibility of getting accurate and verifiable stimulations of this structure is not great. The results obtained in this series of experiments are graphically expressed in the figure. In occasional trials which were made to test the effect of caudate inhibition upon reflex activity it was uniformly found that whenever inhibition of spontaneous activity was obtained there also

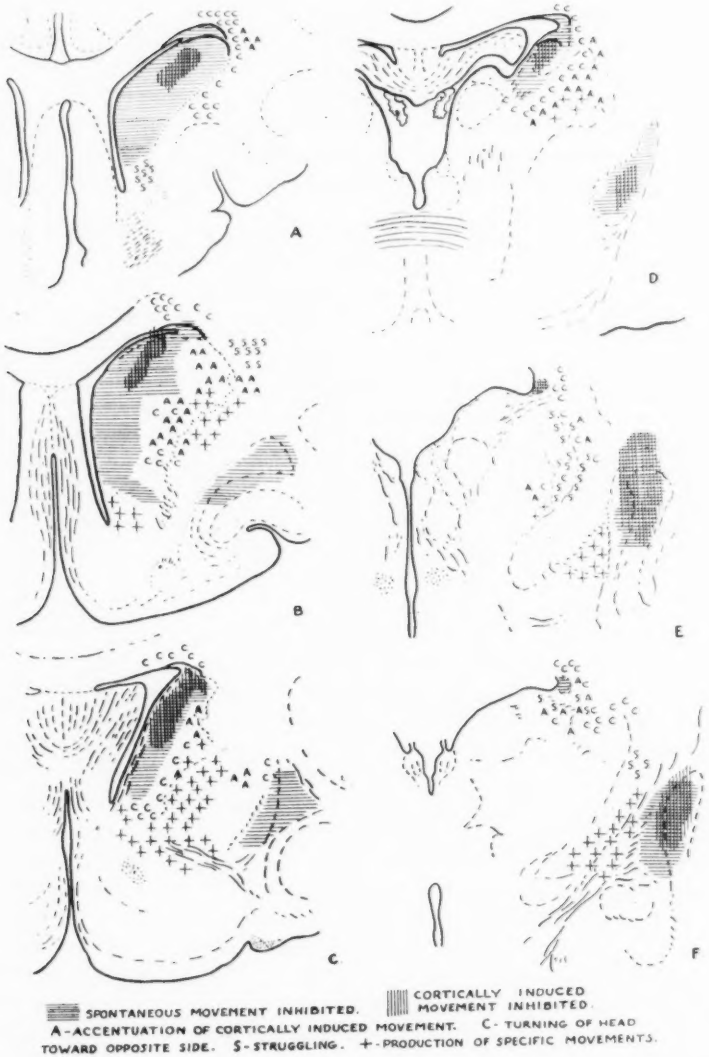

Fig. 1. Diagrammatic representation of results obtained upon stimulation of the feline neostriatum and its vicinity.

occurred an inhibition of the usual, easily-elicitable, normal reflexes, including withdrawal from a painful stimulus.

Objections against attribution of inhibitory function to caudate nucleus from preceding data. The following may be offered against the assumption that the preceding evidence indicates an inhibitory function on the part of the caudate nucleus. In the first place it may be questioned whether the inhibition of movement is not the result of a "diversion of interest" subsequent to irritation of the ventricular ependyma or stimulation of certain of the sensory radiations issuing from the thalamus. Again it will be observed that head-turning toward the opposite side is frequently obtained and it might be supposed that the inhibition might be due to a sub-threshold tendency to offset the normal muscular balance such as is incident to head-turning in which case well-known inhibitory mechanisms (such as the vestibulospinal) are activated. It might also be objected that inhibitory fibers, crossing in the callosum from the opposite side, might be the really responsible agents.

In our previous report we sought to meet the possible objection of current spread by fulgurating at the tip of the electrode and then restimulating. In such cases it was always found that fulguration of the small area at the tip of the electrode abolished the responses which we were recording. The objection of current spread can be also, to some degree, met by the following experiment.

Ten cats were prepared by removing all cortex anterior to and including the posterior sigmoid gyrus. Under such circumstances the thalamoparieto-frontal link is broken, the possibility of stimulating sensory cortical areas is removed and the chance of activating a possible callosal inhibition is reduced. When this was done it was noticed that a greater area of the caudate (in the direction of the internal capsule) was found to be inhibitory. In such a case the electrode is even farther away from the ependyma than previously. This would indicate that the thalamo-cortical fibers tend to mask (by introducing extraneous struggling) and not enhance inhibition. It is to be noted that sensory stimulation usually causes an increase in respiration, while, in the great majority of the experiments reported above, not only was this absent but inhibition (usually of both the frequency and amplitude of the movement) occurred.

Another more serious objection may be raised against the above experiments. It is by no means impossible that fibers of passage in the internal capsule and arising from cortex which is inhibitory may have been stimulated. In order to eliminate this possibility the following experiments were devised.

Stimulation of caudate nucleus in cats from which frontal cortex was previously removed. The length of time required for the dissolution of central neural tracts to the point where they are non-conducting probably varies from system to system. Our own evidence indicates that pyramidal fibers cease to transmit impulses on the fourth day after decortication. This is the same length of time which we found to be required for the severed brachial plexus to become non-conducting. Measurements made on the second day after section of such nerves have shown no perceptible change in conductility. On the third day they show impairment in the threshold of excitability and on the fourth are totally inexcitable throughout their entire length. So far as we know the most resistant nerves after section are the sympathetics, which Gibson found to become non-conductile in eight days.

Eight cats were operated under aseptic precautions and all neocortex anterior to and including the posterior sigmoid gyrus was removed bilaterally by means of the ligature and suction technique, care being used to avoid opening the ventricle and infringing upon the caudate itself. The olfactory tracts were eliminated. The animals were reoperated as follows: two on the fourth postoperative day, one on the fifth, one on the twelfth, two on the thirteenth and two on the sixteenth. In all cases preliminary exploration of the edge of the remaining cortex with the bipolar electrode was without a motor effect and, in every case, stimulation of the caudate

or its vicinity (placements were verified, of course, after death) produced inhibition of spontaneous movement (and often respiration), without evidence of spasticity or rigidity, whenever such movements were present. Stimulation of such portions of the thalamus as lie adjacent to the caudate usually produced turning of the head toward the opposite side, or, if deeper, a rage-like reaction with rapid, phasic clawing and spitting.. Stimulation of adjacent portions of the corona radiata or internal capsule either produced no effect, or resulted in inhibition of spontaneous movement. Stimulation of the fragmentary corpus callosum produced no discernible effect. Head-turning was noticed in thirteen and sixteen-day animals as well as earlier ones (in which it is conceivable that complete non-conductility might not have occurred) and is thus presumably not necessarily dependent upon the presence of the frontal cortex nor that part of the callosum concerned with this area. Accidental evidence that this movement does not emanate from the caudate and is not responsible for such inhibition as we have observed is provided by the case of an animal in which the heads of both caudate nuclei were inadvertently removed. This cat showed no trace of inhibition but head-turning was obtained when the placements approached the thalamus. Thus it would appear that this form of head-turning emanates from the thalamus. Moreover this animal provides independent negative evidence that the inhibition which is customarily obtained cannot be elicited if the striatum is removed.

That inhibition and head-turning (and also tail-switching) can occur through routes other than passage over the pyramids we have found in six animals in which both pyramids were cut. In a previous paper (2) we have already remarked upon the occurrence of inhibition following cortical stimulation after pyramid section and may now compare the effects of caudate and cortical stimulation in preparations of this type.

Comparison between inhibition obtained from caudate and cortex following pyramid section and effect of caudate narcotisation and removal. In the communication referred to above we mentioned that we have had the opportunity to verify the observation of Tower and Hines (3) (see also Tower 4, 5)) that stimulation of the frontal cortex, following pyramid section, results in inhibition of spontaneous movement. In practically all respects our results are in agreement with theirs, the only discrepancy being that while at one time or another they seem to have believed stimulation of the entire excitable area produces inhibition after pyramid section we have found the medial third of the dorsal aspect of the anterior sigmoid gyrus to be most effective in this respect and, when discriminating methods of stimulation were employed, frequently encountered no inhibition from any other portion of the motor region. There is little doubt that the effect which Tower and Hines described is a genuine neural phenomenon of first importance.

That the inhibition obtained upon cortical stimulation and caudate activation may be really identical is, of course, a ready conjecture but before it can be given serious consideration it is necessary to prove that inhibition of spontaneous activity by caudate stimulation, following pyramid section, is possible. In six cases of bilateral pyramid section such a possibility was verified. Cortical stimulation, producing inhibition, in these animals was believed to be generally less effective than deep

stimulation. In one animal in which one pyramid (left) only had been cut it was found that stimulation of the left cortex exercised an inhibitory effect over movements initiated in the right. In three of the above bilateral cases the caudate was narcotised by thrusting a long needle (26 gauge), carried in the Horsley-Clarke instrument, into its head. Into this 0.025 cc. of 20 per cent novocaine was then injected. This resulted in an abolition of the cortical inhibitory effect.

Since, however, it is difficult to tell whether or not this procedure produced a non-conduction of the fibers of projection outside the caudate the following experiment was tried.

At 3:50 p.m. the left caudate of an otherwise normal cat, in which the cortices had been exposed, was injected as above. The left cortex was stimulated at intervals of a minute and was found to be growing more excitable. At 3:55 stimulation of it resulted, according to our notes, "In very loose and floppy movements of the right leg; these movements have a very active tremorous factor in them and have a tendency to display after-discharge, when stimulation has ceased." At 4:00 p.m. the same result was obtained and the right leg was noted to respond (upon stimulation of the left cortex) "more promptly and energetically than the left." At 4:05 it is noted that the left cortex was giving movements which were more dystonic than before but was still more sensitive than the right. At 4:10, more novocaine was injected and this note appears (whether the novocaine was injected before or after the cortex was stimulated we do not know), "there is a marked tendency for after-discharge to develop from [stimulation of] the left cortex and a somewhat smaller tendency for it to develop on [from stimulation of] the right [cortex]. . . . There is some increase in extensor hypertonia on both sides. No apparent difference between the legs."

This procedure was repeated in four other animals. In each case the cortex of the hemisphere in which the caudate was injected became more excitable (its stimulation threshold decreased), after-discharge was noticed and it was observed that there was a greater incidence of spontaneous activity than before. Injection of the caudate with strychnine did not seem to produce any very definite result it merely being observed that it seemed as though the corresponding leg was working against a greater "resistance" than was usual.

While the injection of a substance such as novocaine can hardly be expected to have given a localized narcosis of the caudate, this doubtless had received the major effect of the dose. It would appear that the cortex had been released by this procedure and that the abolition of cortical inhibition after pyramid section and caused by narcotisation of the caudate was not the result of anesthetizing fibers in the internal capsule but it may be interesting to see what can be done by mechanical ablation, sparing the cortex as much as possible.

Stimulation of the cortex following ablation of the caudate nucleus. Adult cat (1-11-CC 6). Cortex exposed. Anterior half of right hemisphere removed and, after elevation of the left half of the corpus callosum, an attempt to remove the left caudate nucleus through the ventricle was made. (Postmortem examination showed

that a small piece of the posterior portion of the head had escaped ablation.) Stimulation of the left anterior sigmoid gyrus now produced rapid, lightning-like thrusts of the leg. These had a loose, dangling character: the leg flying up into the air and collapsing into flabby, pendular movements. Sometimes these movements went into a tonic flexion pattern in which the leg was held up under the mandible. The movements, on the whole, looked like those obtained from acerebellar animals. No evidence of inhibition could be observed.

Adult cat (1-12-CC 7). Cortex exposed and the ventricles on both sides were opened by two cuts made perpendicularly to the falx cerebri, and on either side of it, about half-way back on the brain. An attempt was now made to remove both caudate nuclei through the ventricles thus exposed. Stimulation of the right cortex gave no response. (Postmortem examination revealed that, on the right, the suction tip entered into the internal capsule and disrupted it. On the left, the anterior and medial portions of the head of the caudate only had been successfully removed.) Stimulation of the left cortex produced two different varieties of results. One type of these consisted in a flash-like bat which ended in tonic extension. The other consisted of loose, pendular movements, of a phasic character, which were thrown about by a quick tremor. Movements elicited from the medial aspect of the anterior sigmoid gyrus did not appear to have any tendency to develop hypertonic manifestations whereas stimulation of the lateral part of the gyrus frequently produced extensor, hypertonic thrusts. These characteristics held for all stimulation frequencies from 40 to 750 pulses per second. Frequencies below 40 gave only jerky movements in the extremity. No evidence of inhibition could be observed.

Here again then striatal dysfunction abolished the inhibitory capacity of the cortex without eliminating its positive motor effect which, on the contrary, was accentuated.

Putamen. Stimulation of the putamen was carried out in the same manner as that of the caudate. The series studied consisted of twenty verified placements in the putamen-claustrum complex. The results obtained are indicated in the accompanying figure. Briefly, it was found that the more lateral portions of the complex gave inhibition of both cortically induced and spontaneous movements; that stimulation of the pallidal margin of the putamen resulted in specific movements and that, between these areas, only spontaneous movement could be satisfactorily inhibited. The explanation of the production of movements when the pallidal margin is stimulated is bound up with the function of the globus pallidus which forms the subject of a separate investigation.

Of course, we are again faced at this juncture with the question of the possible rôle which fibers of passage may play in the inhibitory phenomenon. It is not easy to eliminate these in the case of the putamen but at least the same procedure may be adopted as was employed in the case of the caudate. From four animals the excitable cortex was accordingly removed and, ten days later, the putamen was stimulated. Inhibition of spontaneous activity was still obtained and seemed somewhat more effective than in the intact animal. In another animal from which the anterior third of the brain had been removed, including the heads of the caudate nuclei, it was found, twelve days postoperatively, that stimulation of the putamen similarly inhibited spontaneous activity. Such inhibition was also obtained in one animal

after section of the pyramids just below the pons. While we have not subjected the putamen to as exhaustive a study as the caudate and while it is difficult to discuss the effects of lesions made in it without also considering pallidal function there seems to be, from what evidence is here presented, no reason to suppose that it functions in a manner essentially different from the caudate.

CONCLUSIONS

1. Stimulation of the caudate nucleus results in more marked inhibition of movements elicited by stimulation of the cortex of the same than opposite side.

2. In the case of spontaneous movements caudate stimulation exerts a general, bilateral inhibitory effect which is essentially the same on both sides of the body.

3. Spontaneous activity is more easily inhibited than is cortically induced movement and is obtained from a greater area of the striatum.

4. Those areas from which inhibition of cortically induced movement is most easily evoked coincide rather closely with the regions through which corticistriate fibers run in closely grouped bundles. The most notable of these bundles is the subcallosal fasciculus.

5. No evidence favoring the existence of a definite somatotopical projection of the striatum was observed.

6. The inhibitory effect obtained upon striatal stimulation is not related to irritation of the ventricular ependyma, stimulation of the callosum or to excitation of thalamocortical or corticifugal fibers travelling in the internal capsule.

7. Inhibition of the above type cannot be evoked by stimulation in the vicinity of the head of the caudate if this is removed.

8. The inhibition of movement by cortical stimulation as previously discovered by other observers is abolished by narcotisation or injury of large portions of the striatum but is not affected by pyramid section. This would seem to indicate that the cortical inhibitory effect travels through the striatum.

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THE INTERRELATION OF OXIDATIVE AND GLYCOLYTIC PROCESSES AS SOURCES OF ENERGY FOR BULL SPERMATOZOA[1]

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Although work on the respiration of spermatozoa has been reported, investigators are not in agreement as to the significance of the oxidative processes, and the nature of the substances oxidized has not been established. Redenz (1) found that various carbohydrates promoted motility only if the sperm could produce lactic acid from them. He also showed that lactic acid had no influence on motility, but it greatly increased the respiration of spermatozoa in dialyzed serum (1). The lactic acid which accumulates in semen during storage does not account for all of the glucose that disappears (2, 3).

Evidence that oxidative processes promote motility was obtained by Redenz (1) who showed that in a sugar-free medium, spermatozoa soon became immotile when kept under nitrogen but retained motility in the presence of oxygen.

In recent studies on the dehydrogenase activity of spermatozoa (as measured by the Thunberg technique) we observed that the methylene blue reduction time was greatly prolonged in the presence of glucose (4). This appeared to be an inhibition or sparing of oxidative processes by glucose.

In the present work it was our purpose to determine the nature of the intracellular substances other than glucose which are normally utilized for energy by bull spermatozoa and to study the relationship between the oxidative and glycolytic processes.

METHODS. The methods used for semen collection, motility observations and lactic acid determination, and the buffer solution in which the spermatozoa were suspended for motility and respiration studies have been previously described (4). Phosphorus was determined by the method of Fiske and Subbarow (5) using the Evelyn photoelectric colorimeter (6) to obtain the intensity of the color developed. Phospholipids were sepa-

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rated by Bloor's method (7). Aliquots of this alcohol-ether extract were evaporated to dryness, wet ashed with H_2SO_4, the charring which accompanied the ashing cleared by the addition of a few drops of HNO_3, and the inorganic phosphorus determined. Oxygen uptake was measured in air at 37° in the Barcroft apparatus. The center cups of the respiration flasks contained strips of porous filter paper saturated with 20 per cent KOH to absorb CO_2. A 5 minute equilibration period was allowed before measurements were begun.

RESULTS. The results in table 1 demonstrate clearly that when the spermatozoa were separated from the nutrients present in the seminal fluid motility was retained only under aerobic conditions. Added glucose

TABLE 1

Effect of glucose on motility of spermatozoa under aerobic and anaerobic conditions

SOURCE OF SPERMATOZOA	MEDIUM, RINGER-PHOSPHATE PLUS	ATMOSPHERE	MOTILITY RATING			
			½ hour	1 hour	2 hours	3 hours
Bull A	None	Air	5+	2+	1+	Few motile
	None	Nitrogen	Few motile	Dead		
	0.02 M glucose	Air	5+	5+	3+	2+
	0.02 M glucose	Nitrogen	5+	5+	2+	2+
Bull B	None	Air	4+	2+	2+	1+
	None	Nitrogen	Few motile	Dead		
	0.04 M glucose	Air	5+	5+	4+	4+
	0.04 M glucose	Nitrogen	5+	5+	5+	4+

Spermatozoa were centrifuged from the semen, washed by suspending in 0.9 per cent saline and after centrifuging again were suspended in sufficient Ringer-phosphate buffer (pH = 6.8) to give a final volume twice that of the original semen. Incubated at 37°.

promoted motility anaerobically and prolonged it aerobically. Since spermatozoa in the Ringer-phosphate medium containing no sugar remain motile only in the presence of air, it seems that oxygen is required for the utilization of the intracellular reserves of the sperm cell.

The production of lactic acid from various sugars by spermatozoa from 2 different bulls is shown in table 2. Only those sugars which the spermatozoa could catabolize to lactic acid were effective in maintaining motility. Since washed spermatozoa have been shown to contain a small amount of glucose (8), it is probable that this was the source of the small amount of lactic acid produced in the absence of added sugar. Undoubtedly the small amount of motility found initially under anaerobic conditions (table 1) results from the glucose present in the spermatozoa.

Chemical changes in semen during storage. In the hope that changes

in the chemical composition of semen during storage might give some evidence as to the type of substances utilized by spermatozoa, several analyses of semen, before and after storage, were made. It was found that during storage there was a decrease in glucose, an increase in lactic acid, ether extractable substances and ester phosphorus, a very slight increase in non-protein-nitrogen and a considerable decrease in lipid phosphorus.

TABLE 2

Utilization of various sugars by spermatozoa

MEDIUM, RINGER-PHOSPHATE. SUGAR ADDED	MOTILITY AT 2 HOURS	LACTIC ACID PRODUCED BY 1 CC. SPERM SUSPENSION* IN 2 HOURS	
		Bull A	Bull B
		mgm.	*mgm.*
None	Few motile	0.061	0.051
Glucose	4+	0.66	0.59
Maltose	4+	0.66	0.52
Fructose	4+	0.59	0.61
Mannose	4+	0.54	0.52
Galactose	Few motile	0.11	0.14
Sucrose	Few motile		0.12

* Sperm concentrate: Approximately 600 million/cc.

Semen was centrifuged and the spermatozoa suspended in Ringer-phosphate, pH = 6.8. All sugars added to give a final concentration of 0.04 M in the suspension. Incubated at 37° aerobically.

TABLE 3

Typical changes in phosphorus partition of semen during storage at 10°C.

SAMPLE	INORGANIC P	TOTAL ACID-SOLUBLE P	ESTER P	LIPID P
	mgm./cc.	*mgm./cc.*	*mgm./cc.*	*mgm./cc.*
Original	0.012	0.170	0.158	0.276
Stored 56 hours	0.013	0.236	0.223	0.098

Typical results of changes in the phosphorus partition which occurred during storage are shown in table 3. The large decrease in lipid phosphorus was accompanied by an increase in the ester phosphorus fraction. These changes in the phosphorus partition occurred more rapidly at room temperature than refrigerator temperature. If the semen sample was subjected to heat at 80°C. for 5 minutes prior to storage, changes in the lipid phosphorus did not occur. Figure 1 demonstrates that the decrease in lipid phosphorus occurred largely during the first 24 hours of storage and that it paralleled the degree of motility. The decrease in lipid phosphorus

occurred also in spermatozoa separated from seminal fluid and suspended in Ringer-phosphate buffer.

Sparing of oxidative processes by glycolysis. Table 4 shows the effect of glucose on the respiration of spermatozoa. It is seen that the "endogenous" respiration is much greater than the respiration in the presence of glucose. The differences were greatest during the first part of the period, but the rate of oxygen consumption continued, for some time, to be greater in the absence of glucose. Thus glucose has a sparing action on the respiration of spermatozoa. This sparing action seems to depend

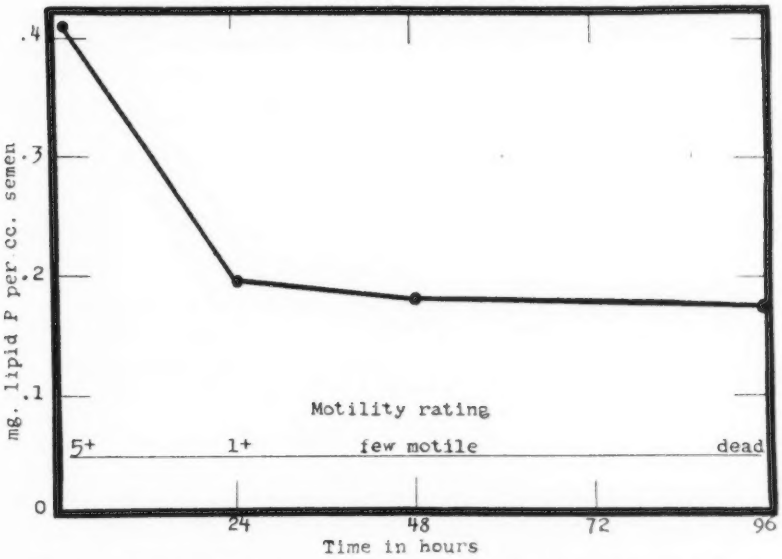

Fig. 1. Changes in phospholipid content of semen during storage at 10°C. aerobically.

on the previous treatment given the spermatozoa. Considerable decreases in oxygen consumption were obtained when glucose was added to fresh, rapidly respiring spermatozoa which had been centrifuged out of the seminal fluid and made up directly in Ringer-phosphate buffer. With certain samples washing lessened the sparing action of glucose on respiration, and occasionally, especially in old samples, glucose even increased the oxygen uptake above the "endogenous" rate. A plausible explanation is that in the older samples many of the intracellular reserves were already oxidized, and the addition of glucose yielded lactic acid, some of which was oxidatively removed.

It seems that the oxygen uptake of bull spermatozoa can be the result of the oxidation of two types of substances: the intracellular reserves and, as shown by Redenz (1), the lactic acid produced by glycolysis. From our studies it is apparent that the endogenous respiration resulting from the oxidation of intracellular reserves is a source of energy for the main-

TABLE 4

Effect of glucose on the respiration of spermatozoa

SOURCE OF SPERMATOZOA BULL	SPERM COUNT	MOLARITY OF ADDED GLUCOSE	CU. MM. OXYGEN CONSUMED PER HALF-HOUR							
			30 minutes	60 minutes	90 minutes	120 minutes	150 minutes	180 minutes	210 minutes	Ave.
	billion/flask									
G*	5	0.00	62.5	32.0	29.3	30.0	24.2			35.6
		0.01	29.9	29.6	25.5	26.0	24.0			27.0
A*	2	0.00	22.4	12.7	10.0	9.8				13.7
		0.02	10.8	10.8	7.0	6.4				8.7
K*	.23	0.00	34.9	22.1	15.0					24.0
		0.02	28.0	19.2	17.8					21.7
B*	.25	0.00	40.0	30.0	20.1	17.9	17.0	16.5	15.4	22.4
		0.04	17.5	16.5	16.2	13.8	12.5	11.5	11.5	14.2
K†	.23	0.00	23.0	12.0	10.0	10.0	9.0			12.8
		0.02	17.5	17.0	10.5	10.0	9.0			12.8
G†‡	3	0.00	13.0	11.3	15.7	15.5	17.0			14.5
		0.02	26.0	21.0	15.0	13.0	12.0			17.4

All Barcroft flasks contained 2 cc. sperm suspension made up to a final volume of 3 cc. with Ringer-phosphate buffer, pH = 6.8. Suspensions prepared as indicated:

* Semen centrifuged and spermatozoa suspended in sufficient Ringer-phosphate to give original volume.

† Spermatozoa were centrifuged from semen, washed by suspending in 0.9 per cent saline and after centrifuging again were suspended in sufficient Ringer-phosphate medium to give original volume of semen.

‡ Specimen stored 2 hours at room temperature and 7 hours at 10° before the experiment was begun.

tenance of motility in bull spermatozoa since in the absence of glycolyzable sugars motility is not retained under anaerobic conditions.

The decrease in oxygen consumption obtained when glucose was added indicated that the sperm shifted to glycolytic mechanisms for some of their energy; however, the magnitude of the decrease is not a true criterion of the degree of the shift. Even though the spermatozoa were obtaining

all of their energy from glycolysis, a residual oxygen uptake would continue because of the oxidative removal of part of the lactic acid produced. It must be remembered that the energy released by the oxidation of lactic acid is apparently not available to the sperm for the maintenance of motility, for Redenz (1) found that added lactate increased the respiration but did not increase motility of sperm suspensions containing no sugar.

Table 5 shows that there was very little decrease in the phospholipid content of spermatozoa when glucose was added to the suspension medium. This, together with the sparing action of glucose on respiration, is an indication of the preferential utilization of glycolytic mechanisms by spermatozoa as a means of obtaining energy.

Discussion. It is apparent from these results that spermatozoa have the ability to utilize a variety of sugars including the disaccharide maltose.

TABLE 5

Effect of glucose on phospholipid changes in spermatozoa

MEDIUM	LIPID PHOSPHORUS	
	Original	After 10 hours' incubation
	mgm./cc.	*mgm./cc.*
Ringer-phosphate	0.38	0.24
Ringer-phosphate-glucose	0.39	0.37

Semen was centrifuged, the spermatozoa made up to original volume with Ringer-phosphate and incubated at room temperature. Final glucose concentration was 0.04 M.

At present we cannot say whether the inability of sperm to utilize galactose and sucrose is the result of an impermeability of the sperm to these sugars or the lack of enzyme systems capable of utilizing them.

While the decrease in lipid phosphorus during storage certainly is not final evidence that phospholipids are being utilized by spermatozoa, the fact that the decrease may be lessened by adding glucose to the sperm suspension gives support to the probability that in the absence of glucose the spermatozoa are using intracellular phospholipids as a source of energy for motility. The mechanism of this utilization is not known. A possible explanation is that the phospholipids are hydrolyzed, perhaps liberating most of the phosphorus as an acid soluble ester (table 3) and the fatty acid portion may then be oxidized to obtain energy. The possibility that the decrease of phospholipid during storage is the result of enzymatic hydrolysis unrelated to any metabolic needs of the sperm is lessened by the following considerations. First, in the absence of sugars the quantity of phospholipid that disappears parallels the degree of motility of the sperma-

tozoa. Second, the disappearance of phospholipid can be lessened by adding glucose to the suspension medium. Finally, in the presence of glucose spermatozoa depend less on oxidative processes for their energy.

Spermatozoa are not unique in showing a decrease of respiration in the presence of glucose. This phenomenon has been observed in certain tumors (9, 10, 11), in lymph nodes of leukemic mice (12) and in bovine articular cartilage (13). The cause underlying the decrease of respiration in the presence of glucose has not been determined. Crabtree suggested that the glycolytic activity of tumors may act as a partial check on their respiratory powers. Rosenthal et al. (13) obtained the effect with both glucose and mannose but not with fructose which is not glycolyzed by cartilage. They offered the explanation that added glucose is oxidized slower than the cellular substances "the oxidation of which it replaces" (13). On the basis of our results we offer the following explanation. In spermatozoa the energy requirement for the maintenance of their vital activity can be obtained from the oxidation of intracellular substances or from glycolytic processes. When sugars are available to the spermatozoa the energy obtained from their breakdown to lactic acid lessens the demand on the oxidative processes.

SUMMARY

1. In confirmation of the observation of Redenz it has been shown that, in a medium containing no sugar, spermatozoa remained motile only in the presence of air, indicating that oxygen was required for the utilization of the intracellular reserves. Added sugars maintained motility only if they could be catabolized to lactic acid by the spermatozoa.

2. During storage the phospholipid content of semen decreased. The decrease occurred also in sperm suspensions free of seminal fluids and here could be lessened by the addition of glucose. The decrease in phospholipids paralleled the oxidative utilization of intracellular reserves for the maintenance of motility.

3. The rate of respiration by spermatozoa in the presence of glucose was much less than the "endogenous" rate which indicated a preferential utilization of glycolytic mechanisms as a means of obtaining energy for motility.

From these studies it is concluded that phospholipids are the source of the intracellular reserve energy of bull spermatozoa, that this energy is obtained by oxidative processes, and that spermatozoa preferentially obtain the energy for motility from the glycolysis of glucose or other glycolyzable sugars.

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AGE CHANGES AND SEX DIFFERENCES IN ALVEOLAR CO_2 TENSION[1]

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The alveolar CO_2 tension has been shown to be higher in males than in females (4) (5), a finding confirmed by a corresponding difference in pCO_2 of arterial blood (13). The aim of the present report is to determine the age at which the sex difference in the alveolar CO_2 tension first appears, the extent of the difference, and the possible explanation for it.

EXPERIMENTAL. *Subjects.* Determinations of the tension of CO_2 in the alveolar air were made on fifty normal boys and fifty normal girls from Oakland, California, school children. The children were first tested when they were between the ages of 11 and 12 years (mean 11.87; S.D. 0.5 year) and were re-tested at six-month intervals over a six-year period. Duplicate observations on each subject under basal conditions were carried out on two successive days. After a fifteen-minute rest period in the supine position, the first sample of alveolar air was collected at the end of expiration in the sampling valve described by Henderson and Morriss (8) and transferred immediately to gas sampling tubes over mercury. A second sample of alveolar air was obtained in the same manner at the conclusion of a basal metabolism test. Analysis for CO_2 content was made in duplicate on a 10 cc. Haldane gas analysis apparatus. The CO_2 tension was computed on the basis of the barometric pressure taken at the time the sample was obtained. The average of the two samples for each day was computed as well as the average of all measurements for all subjects at each age level.

Determinations of alveolar air pCO_2 were made in the same manner as described above on additional groups of subjects as follows:

Adolescent groups A and B. These groups consisted of fifty normal boys (A) with a mean age of 16.0 ± 0.2 and fifty normal girls, with a mean age

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of 16.0 ± 0.2, also from the Oakland schools. These children were selected on the same basis as the original group tested and differed only in that they had never been subjected to the physiological testing program before, in contrast to the other children measured, who had been tested eight times previously by the time they had reached a similar age.

Adult group. Measurements were made on a group of twenty male students, with a mean age of 23.9 ± 0.7 (range 20–26 yrs.) and fifteen female students with a mean age of 23.2 ± 0.7 (range 19–26). Values for alveolar pCO_2, under the same conditions, were obtained on twenty adult males, mean age 32.5 ± 1.3 (range 27–43 yrs.) and seventeen adult females, mean age 33.4 ± 1.2 (range 27–39 yrs.). The latter groups were chosen largely from staff members.

TABLE 1

Reliability of pCO_2 determination in adolescents probable error of a single test (mm. Hg)

TESTING	DATE OF TESTS	AGE YEARS (Mn)		PROBABLE ERROR BASED ON			
				2 tests—same day		Test on 2 days	
		Boys	Girls	Boys	Girls	Boys	Girls
I	Spring '33	11.9	11.9	1.3	1.2	1.4	1.4
II	Fall '33	12.5	12.5	1.4	1.6	1.5	1.6
III	Spring '34	12.9	12.9	—	—	1.2	1.3
IV	Fall '34	13.5	13.6	1.9	1.7	1.6	1.7
V	Spring '35	14.0	13.9	—	—	.9	1.5
VI	Fall '35	14.6	14.5	1.4	1.6	1.6	1.8
VII	Spring '36	14.9	14.9	—	—	1.3	1.5
VIII	Fall '36	15.5	15.5	1.9	1.6	1.9	1.5
IX	Fall '37	16.5	16.5	1.7	1.5	1.5	1.6
X	Fall '38	17.5	17.4	1.5	1.6	1.3	1.6
I	Adolescent groups A and B	16.0	16.0	1.6	1.6	1.6	1.4

RESULTS. *Reliability of measurements.* Since two determinations of alveolar air were made on each of the two experimental days, it is possible to calculate the probable error of the determination[2] based on either single observations on a given day or on the basis of the mean of two observations made on different days. The results of this analysis are shown in table 1. It is clear that the probable error for a single determination of

[2] The formula used for this calculation was:

$$P.E._{.1_{\infty}} = \left[0.6745 \frac{\sigma_1 + \sigma_2}{2} \sqrt{1 - r_{11}}\right] \quad \text{(Garrett (6), p. 321)}$$

Where $P.E._{.1_{\infty}}$ is the probable error of the score; σ_1 is the standard deviation of the distribution of the first test; σ_2 is the standard deviation of the distribution of the second test and r_{11} is the coefficient of correlation between the tests.

alveolar CO_2 tension is about 1.5 mm. It also appears from this table that the error is slightly greater for girls than for boys.

Age changes. From the average of determinations made on two days, distributions were made at each age level. Class intervals were arranged so that the mid-points fell at 11.5, 12.0, 12.5, etc., years. Since the testing interval could not be maintained at exactly six months for each child throughout the study, the number of cases varies from 50 to 32 at each age category except for 11.5 years and 17.5 years, where the number falls as low as 16. If two tests on the same child fell within the same age

TABLE 2

Age changes and sex differences in basal alveolar CO_2 tension

AGE MID-POINT	ALVEOLAR pCO_2 — MM. Hg						
	Males		Females		Difference Male − Female	S.D.diff.	Mn diff. / S.D.Mn diff.
	Mn	S.D.Mn	Mn	S.D.Mn			
11.5	40.5	0.74	40.6	0.64	−0.1	0.98	0.1
12.0	41.0	0.50	40.1	0.36	0.9	0.61	1.5
12.5	41.4	0.38	40.3	0.38	1.1	0.54	2.0
13.0	42.5	0.33	39.8	0.42	2.7	0.53	5.1
13.5	42.0	0.33	39.6	0.37	2.4	0.49	4.9
14.0	42.2	0.35	39.4	0.47	2.8	0.58	4.8
14.5	42.3	0.37	39.6	0.41	2.7	0.55	4.9
15.0	42.1	0.41	39.0	0.41	3.1	0.58	5.3
15.5	42.4	0.41	38.6	0.52	3.8	0.66	5.8
16.0	42.3	0.58	38.8	0.43	3.5	0.72	4.9
16.5	44.0	0.34	39.9	0.49	4.1	0.60	6.8
17.0	44.2	0.42	40.1	0.51	4.1	0.66	6.2
17.5	44.7	0.40	40.4	0.61	4.3	0.73	5.9
16.0*	42.4	0.42	40.2	0.60	2.2	0.73	3.0
24.0	43.0	0.69	41.6	0.94	1.4	1.16	1.2
33.0	42.7	0.84	40.0	0.75	2.7	1.12	2.4

* Mean values for group of 16 year-old children without previous test experience

category only one test (the one nearest the mid-point of that age category) was used in computing the age norms shown in table 2. The data show a statistically significant rise in alveolar CO_2 tension in boys between the ages of 11.5 and 17.5; no significant change in alveolar CO_2 tension in girls was observed over the same age range (14).

Relationship between alveolar pCO_2 and body size. The surface area for each subject was computed from height and weight by the Du Bois formula (3). Each pCO_2 value was then divided by the surface area and mean values for each age interval were calculated as before. The results are shown in table 3 from which it may be seen that with such an adjust-

ment for body size the values of alveolar pCO_2 per square meter of surface area for males and females are not significantly different beyond the age of 15 years.

A similar analysis was made in which the alveolar pCO_2 was divided by body height. This adjustment for size also resulted in similar values for males and females (pCO_2 in mm.Hg per cm. height).

TABLE 3

Age changes and sex differences in basal alveolar CO_2 tension adjusted for body size

AGE YEARS	ALVEOLAR pCO_2 — MM. Hg PER SQ. M. SURFACE AREA						
	Males		Females		Difference Male − Female	$S.D._{diff.}$	Mn diff. / $S.D._{Mn\ diff.}$
	Mn	$S.D._{Mn}$	Mn	$S.D._{Mn}$			
11.5	33.3	0.76	31.3	1.00	2.0	1.26	1.6
12.0	32.5	0.56	30.2	0.58	2.3	0.81	2.8
12.5	31.8	0.51	29.4	0.50	2.4	0.71	3.4
13.0	31.6	0.49	28.2	0.46	3.4	0.67	5.1
13.5	29.6	0.48	27.1	0.41	2.5	0.63	4.0
14.0	28.6	0.47	26.1	0.44	2.5	0.64	3.9
14.5	27.6	0.45	25.7	0.34	1.9	0.57	3.3
15.0	26.4	0.45	25.1	0.36	1.3	0.58	2.2
15.5	25.8	0.41	25.0	0.35	0.8	0.54	1.5
16.0	24.8	0.45	24.6	0.37	0.2	0.58	0.3
16.5	25.3	0.35	25.4	0.38	−0.1	0.52	0.2
17.0	25.0	0.37	24.8	0.39	0.2	0.54	0.4
17.5	24.7	0.45	25.6	0.57	−0.9	0.73	1.2
24.0	23.8	0.59	26.1	0.79	−2.3	1.03	2.2
33.0	23.9	0.74	24.4	0.51	0.5	0.90	0.6

TABLE 4

Correlation of body size and alveolar pCO_2

MEASURE OF SIZE	AGE 16.5-17.5 YEARS		
	Boys and girls	Boys	Girls
Height	0.57 ± 0.05	0.21 ± 0.11	0.07 ± 0.11
Weight	0.33 ± 0.07	−0.06 ± 0.11	0.13 ± 0.10
Surface area	0.47 ± 0.06	0.07 ± 0.10	0.10 ± 0.11
Stem length	0.47 ± 0.06	0.07 ± 0.10	0.12 ± 0.10

DISCUSSION. Our results on repeated tests indicate an error of measurement of ±1.5 mm.Hg in alveolar CO_2 tension. Since the error is of the same order of magnitude for tests made at half-hour intervals or one day intervals, this degree of error is attributed to the method of obtaining alveolar air samples. Cordero (2) reported a somewhat greater varia-

bility (±1.5 2.2 mm.) in alveolar air samples taken at three-minute intervals, as did Haldane (7) working with a trained subject.

The age trend in alveolar CO_2 tension which we have found in boys is not in agreement with previous reports by Mori (10) and Robinson (11). However, the number of cases studied by these investigators at each age level was small (3–12 cases per 3-yr.-age group) and none of the subjects was retested over a period of time.

Marriott (9) states that "In infants, the (alveolar) tension of CO_2 is from 3 to 5 mm. lower than adults," but no data are given. Daily observations of alveolar CO_2 tension over a two-year period were reported on the same subject when he was 40 years of age and again when he was 50 years old by Shoji (15) and Sasaki (12). In this Japanese subject the average pCO_2 at the age of 40 years was 36.2 mm. while comparable determinations made ten years later gave an average value of 42.2 mm. Benedict and Root (1) reported alveolar pCO_2 of 46 mm. in a single male, aged 91 years. Some evidence for an increase in alveolar pCO_2 with increasing age is found in the observations of Fitzgerald and Haldane (5), since the average pCO_2 in a group of sixteen boys (ages 8.5–14) was 37.2 mm., while that for a group of twenty-seven males (ages 21–48) was 39.2 mm. Similar differences are reported for a group of females. In our observations a significant age trend in pCO_2 of alveolar air is found in boys, irrespective of any adjustment for size. In the case of girls, an age trend is found only when the values of CO_2 tension are adjusted for size (see table 3).

Evidence for a sex difference in the average values for alveolar CO_2 tension is based on the findings of Fitzgerald and Haldane (5), who found average values of 39.2 mm.Hg for a group of 27 males and 36.2 mm.Hg in a group of 32 females. Similar sex differences were reported by Fitzgerald (4) on individuals living at higher altitudes above sea level. Our results show that a significant sex difference in alveolar CO_2 tension first appears at the age of 13 years. It has also been shown that when adjustment for body size is made, the sex difference disappears after the age of 15, although it is significant between the ages of 12.5 and 14.5 years.

Our observations show that when alveolar CO_2 tension is divided by the surface area of the individual, mean values for the two sexes are the same in adults. If this finding is taken at its face value we might assume that the "sex" difference is in reality nothing more than a "size" difference. If this is true, then large individuals of either sex should show higher alveolar pCO_2 values than smaller individuals of the same sex. In order to test this hypothesis further, the correlation between various measures of size (height, weight, surface area, stem length) and the alveolar pCO_2 was computed for each sex separately. The results are shown in table 4. Since the correlations do not differ significantly from zero for any of the

measures when the sexes are considered separately, we cannot demonstrate that within a sex group of the present sample the alveolar pCO_2 is related to size. Examination of the correlation plots shows that the low correlations obtained may be due to the restricted range of values for size measurements which results when such a homogeneous group is considered. Before concluding that no relationship exists between size and alveolar pCO_2 in a given sex, it will be necessary to make measurements on very large and very small adults of the same sex.

SUMMARY. Determinations of the CO_2 tension of alveolar air sampled by the Haldane-Priestley technique have been made in a group of fifty girls and fifty boys. Two tests were made under basal conditions on each of two successive days. Determinations were made at six-month intervals on each child between the ages of 11.5 and 17.5 years. Average values for boys and girls were computed for each age level. No significant age deviations from 39 to 40 mm. in CO_2 tension were found in girls. In boys, the average alveolar pCO_2 increased from 40.0 mm. at 11.5 to 44.5 mm. at 17.5 years. When an adjustment for body size (height or surface area) was applied to measurements of pCO_2 the sex difference disappeared beyond the age of 15 years. Within a given sex no correlation between size as measured by height, weight, surface area, or stem length could be demonstrated.

CONCLUSIONS

1. The alveolar CO_2 tension of adult males is significantly higher than that of adult females.

2. This sex difference first becomes statistically significant at the age of 13 years.

3. This difference in alveolar CO_2 tension of males and females disappears (in adults) if the alveolar CO_2 tension for each subject is divided by height or surface area.

4. Within a given sex, no significant correlation between body size and alveolar CO_2 tension was found in the present series of observations.

5. Possible explanation for the sex difference must lie in physiological characteristics not present before the age of 13 years.

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THE EFFECT OF THYROID AND CALCIUM THERAPY ON THE SKULL BONES OF THYROPARATHYROIDECTOMIZED RATS

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A roentgenogram of a normal rat's skull reveals a mosaic pattern (1) most marked in the occipital bone and extending less well developed along the mid sagittal suture in the parietal and frontal bones. To a still less extent this configuration may be seen in other parts of the parietal and frontal bones. The normal pattern has been described by Patras (2) as having the appearance of "a calcified network the interstices of which are much less dense."

Thyroparathyroidectomy at an early age is followed by a disturbance in the normal mosaic pattern of the skull bone (3) which has been described as obscured by a decrease in the density of the network.

Oral thyroid therapy (0.02 per cent in the diet) following thyroparathyroidectomy, although capable of producing marked stimulating effects on the growth curve and skeletal size has been found to have no significant influence toward restoring the normal mosaic pattern (3) of the skull bone. This quantity of thyroid though adequate in some respects, may have been inadequate in quantity to alter the details of the configuration. We have therefore studied the effect of 0.05 per cent of desiccated thyroid, preliminary studies having shown that this concentration, administered over a period of 200 days, is well tolerated by rats. The animals (17 females) used for this study were taken from the Loyola colony which is maintained on a diet of Fox Chow *ad libitum* with bread and meat twice weekly. The rats were weaned at the age of 21 days and thyroparathyroidectomized at the age of 50 to 53 days. Immediately after the operation they were given a diet which consisted of Fox Chow 99.95 per cent and 0.05 per cent desiccated thyroid. They received this diet thoughout the course of the experiment which lasted 220 days. At the close of the experiment the animals were killed with ether and the skull bones removed (2) for x-ray study.

A study of the roentgenograms (fig. 1) showed a significant increase in the density of all the skull bones but the blurred condition which obscures

the normal mosaic pattern following thyroparathyroidectomy remained. These results tend to confirm the suggestion made by Patras and Wakerlin (3) that thyroid feeding is capable of increasing the density by a mechanism which permits calcification without maintaining the details of the normal pattern.

Since oral thyroid therapy to the extent of 0.05 per cent in the diet failed to exert an influence toward restoring the normal mosaic configuration of the skull, it seemed advisable to study other methods of altering calcium metabolism. It is obvious from the nature of the operation that the parathyroids may be involved. Since calcium therapy is known to control many of the parathyroid deficiency symptoms it seemed reasonable to anticipate that this procedure might restore the normal pattern to the skull bone of thyroparathyroidectomized rats. At least in conjunction with desiccated thyroid, calcium therapy might be found beneficial.

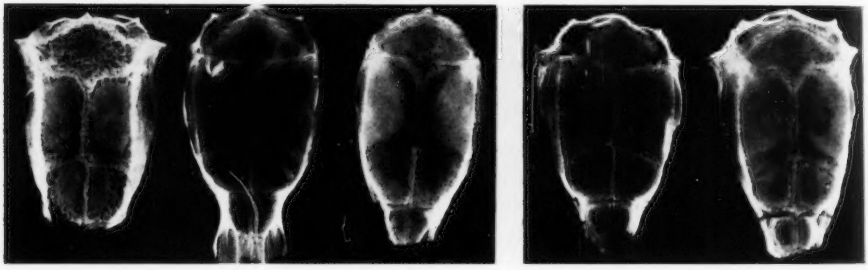

Fig. 1. Skull bones. A, normal rat. B, thyroparathyroidectomized rat. C, thyroparathyroidectomized rat receiving thyroid therapy. D, thyroparathyroidectomized rat receiving added calcium but no thyroid in diet. E, thyroparathyroidectomized rat receiving both calcium and thyroid in diet.

A diet consisting solely of Fox Chow has been found adequate to maintain a colony of rats in a good state of health and reproduction for an indefinite time. The serum calcium and phosphorus of normal rats on this diet was found by Tweedy and associates (4, 5) to average approximately 11 and 5 mgm. per cent respectively. These figures are comparable to those commonly given as representing the calcium and phosphorus content of normal blood serum. Following thyroparathyroidectomy the normal calcium-phosphorus balance is not maintained on this diet. An average serum calcium and phosphorus of approximately 6 and 9 mgm. per cent respectively has been found following thyroparathyroidectomy (6) unless the diet is changed to one high in calcium and low in phosphorus. This inability of the thyroparathyroidectomized rat to maintain a normal calcium-phosphorus ratio in the blood on a Fox Chow diet may account for the blurring of the mosaic pattern in the skull bones. Another possi-

bility is that there is a function of the thyroid, concerned with certain details of bone structure, not obtained through substitution therapy with desiccated thyroid. Finally the parathyroids may be involved either

TABLE 1

GROUPS	DIETS	EXPERIMENT NUMBER	RAT NUMBER	RAT FEMURS		GROUPS	DIETS	EXPERIMENT NUMBER	RAT NUMBER	RAT FEMURS	
				Length	Weight					Length	Weight
				mm.	*mgm.*					*mm.*	*mgm.*
I	Fox Chow	76	1	27.8	347.7	III	Fox Chow	77	1	33.5	602.6
M*	99 and		2	27.3	308.2	M	98.98,	89	1	31.6	563.5
	$CaCO_3$ 1	88	1	27.6	353.7		$CaCO_3$		2	33.0	592.4
			2	29.1	485.1		1.00,		3	35.0	773.6
			3	26.3	312.0		Des.		4	33.2	673.6
			4	26.0	306.4		Thyroid		5	33.0	652.8
			5	26.8	305.8		0.02		6	33.0	684.2
			6	28.0	358.1				7	33.7	602.2
			7	28.3	378.7				8	33.7	706.8
			8	27.9	361.0				9	32.6	565.4
		93	1	26.4	309.0				10	34.5	717.5
			2	29.3	423.2			92	1	34.0	743.3
		99	1	28.0	347.9				2	35.2	818.4
			2	31.6	489.8				3	34.8	610.0
			3	27.3	345.7			98	1	33.7	618.2
			4	27.9	306.0				2	32.8	498.5
		102	1	31.7	487.0			103	1	33.5	591.6
			2	27.8	300.9				2	33.7	602.6
			3	26.4	296.6				3	33.0	569.9
			4	27.8	338.8				4	32.7	519.2
Average				27.9 ±0.2	357.9 ±3.0					33.5 ±0.004	635.3 ±12.4
II	Same as	84a	1	26.8	331.4	IV	Same as	85a	1	29.2	408.6
F	above		2	31.2	421.4	F	above		2	29.3	400.0
			3	26.1	316.8				3	29.9	406.7
		91a	1	25.6	266.6				4	29.7	433.0
			2	25.9	289.4			90a	1	29.6	420.0
			3	26.3	316.0				2	30.4	433.4
		94a	1	27.0	324.0				3	29.7	410.5
			2	26.7	323.8				4	28.6	392.1
		96a	1	26.4	318.9				5	29.3	422.1
			2	27.7	353.0			97a	1	29.0	427.5
			3	24.8	253.0				2	31.0	491.7
			4	26.6	331.4				3	30.8	419.8
		104a	1	26.9	323.9				4	28.9	431.0
		106a	1	26.4	328.9			107a	1	29.5	422.8
			2	27.8	360.4						
Average				26.8 ±0.2	323.9 ±2.1					29.6 ±0.03	422.8 ±4.1

* M, male; F, female.

directly, through their influence on the calcium-phosphorus ratio of the blood, or through some undescribed mechanism dealing with certain details of bone pattern.

To study the hypothesis that calcium therapy in conjunction with

desiccated thyroid might contribute to the restoration of the normal mosaic pattern in thyroparathyroidectomized animals, 69 albino rats from the same colony and treated as previously described were operated between the ages of 26 and 31 days. Following the operation littermates of the same sex and as near the same weight as practical were divided into 4 groups. Groups 1 and 2 (20 males and 15 females respectively) received a diet consisting of 99 per cent Fox Chow and 1 per cent calcium carbonate. Groups 3 and 4 (20 males and 14 females respectively) received a diet consisting of 98.98 per cent Fox Chow, 1 per cent calcium carbonate and 0.02 per cent desiccated thyroid. All animals were kept on their respective diets for 184 days during which time they were weighed at weekly intervals. On the 184th day the animals were killed with ether. The skull bones and left femurs were removed and cleaned for study.

The importance of thyroid therapy following thyroparathyroidectomy is demonstrated in the post operative weight curves (fig. 2). A significant

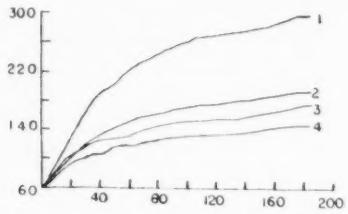

Fig. 2. Weight curves of thyroparathyroidectomized rats.

Group 1, male rats receiving calcium and thyroid in diet. Group 2, female rats receiving calcium and thyroid in diet. Group 3, male rats receiving calcium with no thyroid in diet. Group 4, female rats receiving calcium with no thyroid in diet.

difference in the weight curves favoring the groups which received thyroid was indicated by the second post-operative week. This difference continued at an increasing rate through the succeeding 12 to 14 weeks, after which it remained approximately constant. It is notable that the thyroid therapy seemed to be more beneficial to the males than to the females.

Measurements of the femurs (table 1) following their removal showed a significant difference in length favoring the groups which received thyroid. An average femur length of 27.9 ± 0.2 and 26.8 ± 0.2 mm. was found for the males and females respectively which did not receive desiccated thyroid in the diet. An average femur length of 33.5 ± 0.004 and 29.6 ± 0.03 mm. was found for the males and females respectively in the groups which received thyroid. From these measurements it was found that the average length of femurs taken from the male animals was increased 20 per cent by thyroid therapy while the average length of femurs taken from the females was increased only 10 per cent by thyroid.

The weights of the femurs (table 1) which were obtained after 2 months of drying at room temperature presented an even more significant difference than the lengths displayed. The average weight of femurs taken from the animals which did not receive thyroid was 357.9 ± 3.0 and 323.9 ± 2.1 mgm. respectively for the males and females while the average weight of the femurs from the groups receiving thyroid was 635.3 ± 12.4 and 422.8 ± 4.1 mgm. for the males and females respectively. Thus it was found that the weight of the average femur in the case of the male animals was 77 per cent heavier where thyroid therapy was used while in the case of the females the average femur was only 30.5 per cent heavier

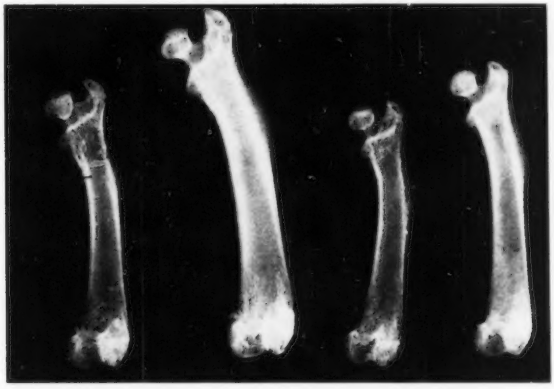

Fig. 3. X-ray pictures of femurs of thyroparathyroidectomized rats.

A, femur of male rat receiving no thyroid. B, femur of male rat receiving 0.02 per cent desiccated thyroid in diet. C, femur of female rat receiving no thyroid. D, femur of female rat receiving 0.02 per cent desiccated thyroid in diet.

in the thyroid fed group. The density and the diameter of the femurs (fig. 3) were considerably greater in the groups receiving thyroid therapy.

Roentgenograms of the skull bones (fig. 1) revealed a blurring of the mosaic pattern previously described (1) as following thyroparathyroidectomy. No significant difference could be seen between the detailed configuration of the skull bones of the two groups, except for an increase in density in the groups receiving thyroid therapy.

From these data it is evident that the addition of calcium carbonate to the extent of 1 per cent to a diet already containing 1.36 per cent calcium, even in association with thyroid therapy, exerts no visible influence toward restoring the clarity of the mosaic configuration in the skull bones of thyroparathyroidectomized rats. The addition of the above quantity of calcium to Fox Chow raised the calcium-phosphorus ratio from approximately 1.4:1 to a ratio of approximately 1.8:1.

Since calcium therapy, alone or combined with thyroid, fails to restore the normal mosaic pattern of thyroparathyroidectomized rats the question of parathyroid therapy becomes most important.

SUMMARY

1. Administration of 0.05 per cent desiccated thyroid in a Fox Chow diet was of no more value than that of 0.02 per cent in restoring the mosaic configuration of the skull bones of thyroparathyroidectomized rats; the bone density, however, was greatly increased in the first case.

2. Raising the calcium-phosphorus ratio in a diet of Fox Chow from 1.4:1 to a ratio of 1.8:1 by the addition of 1 per cent calcium carbonate exerted no significant effect on the mosaic pattern in the skull bones of thyroparathyroidectomized rats.

3. Thyroid therapy (0.02 per cent desiccated thyroid in the diet) in association with calcium therapy (Fox Chow fortified by 1 per cent calcium carbonate) did not lessen the blurring of the mosaic configuration in the skull bones of thyroparathyroidectomized rats.

4. A beneficial effect from thyroid feeding was noticeable in the growth curve and the length of the femurs of thyroparathyroidectomized rats on a Fox Chow diet containing an additional 1 per cent calcium carbonate.

5. The density of skull bones and femurs of thyroparathyroidectomized rats on a diet of Fox Chow fortified with 1 per cent calcium carbonate was increased by the addition of desiccated thyroid (0.02 per cent) to the diet.

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THE RESPONSE OF NORMAL, HYPOPHYSECTOMISED AND ADRENALECTOMISED RATS TO HISTAMINE ADMINISTRATION

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Of the various theories suggested for the etiology of surgical shock, one which has received a considerable amount of attention is the possible liberation of histamine or some closely related substance from the tissues following trauma. The fact that histamine may be extracted from most tissues and the similarity of the changes produced by large doses of histamine to those associated with traumatic shock has supported the idea that a histamine-like substance may be the causative factor in shock. Histamine has an advantage over many experimental surgical methods for producing shock, since it can be injected in graded doses, and strictly comparable studies conducted. In the experiments to be reported, rats have been treated with histamine. Although this species can tolerate relatively enormous doses of histamine, it is especially suitable for studying the effects of hypophysectomy and adrenalectomy. Furthermore, the rat has been frequently used in assay tests for adrenal extracts and corticotrophic hormone. Comparative studies, therefore, have been made on the toxicity of histamine and the effects on blood volume, as indicated by the hemoglobin changes in normal animals, and in those following removal of the adrenals or pituitary gland. The effect of treatment with desoxycorticosterone has been determined in adrenalectomised and hypophysectomised animals, and with corticotrophic hormone in hypophysectomised rats.

METHODS. In most cases adult rats (150 to 200 grams) of a hooded strain maintained in the laboratory have been used. Hypophysectomy or adrenalectomy was performed under ether anesthesia and animals subjected to the latter operation were maintained on 0.9 per cent NaCl instead of drinking water, except where noted. In control experiments approximately 90 per cent of rats of this strain die within 21 days following adrenalectomy without salt treatment. The diet consisted of Purina Fox chow. Histamine dihydrochloride in a 5 per cent solution was given as a single subcutaneous injection. Doses were calculated in terms of body weight and are expressed as the dihydrochloride and not the free base.

Hemoglobin determinations were made on 0.2 cc. of blood drawn from the tail and measured by an Evelyn electric photo-colorimeter. The results are expressed as a percentage of the control value which was obtained for each animal—the control value is also given as grams per cent (calculated as for human blood). The corticotrophic extracts were administered by subcutaneous daily injections. Desoxycorticosterone acetate was injected in a solution of corn oil or made into hard pellets which were implanted into the subcutaneous tissues. In one experiment crystals were similarly implanted.

The corticotrophic extracts used were weak acid hydrolysates of whole pituitaries of cattle partly detoxified by treatment with 1 per cent ammonia on a boiling water bath for one hour. The ammonia was removed by vacuum distillation. Very little success has been had as yet in fractionating these into active and inactive fractions. The corticotrophic potency of different extracts has been related more directly to the total solid content than to any other factor.

RESULTS. *Toxicity of histamine in normal, adrenalectomised and hypophysectomised rats.* The animals used in these experiments were hypophysectomised from 14 to 16 days previously and were in good general condition. The adrenalectomised rats had been operated on from 6 to 8 days previously, and were maintained on 0.9 per cent NaCl. From table 1 it may be seen that normal rats were so markedly resistant to large subcutaneous doses of histamine that even the largest dose tested (1,500 mgm. per kgm.) did not kill the animals. Following hypophysectomy the rats became more susceptible, and a dose of 200 mgm. of histamine per kgm. caused some mortality, while 650 mgm. per kgm. produced death in 85 per cent of the animals. In subsequent experiments to attempt to increase the resistance against histamine, 650 mgm. per kgm. has been used as the test dose. Adrenalectomised rats maintained on saline were also more susceptible to histamine, probably slightly more so than hypophysectomised animals.

Toxicity of histamine in treated hypophysectomised rats. Starting immediately following hypophysectomy, groups of female rats were treated with three different corticotrophic extracts. After 14 to 16 days they received 650 mgm. per kgm. of histamine, by subcutaneous injection, a dose corresponding to that which killed 85 per cent of untreated hypophysectomised animals. Another group of animals received crystals of desoxycorticosterone implanted into the subcutaneous tissue at time of hypophysectomy, and a third group received subcutaneous injections of desoxycorticosterone in oil for 14 days. These results may be seen in table 2. Of the corticotrophic extracts used, no. 278 produced the greatest adrenal enlargement (average weight of adrenals = 42.5 mgm.) and completely protected the animals against the test dose of histamine. The

other less active extracts possibly afforded slight protection. The desoxycorticosterone showed no effect. The crystals which were implanted apparently dissolved rapidly so that after 15 days only traces of them could be found.

TABLE 1

Toxicity of histamine in normal, hypophysectomised and adrenalectomised rats

NUMBER OF RATS	CONDITION	DOSE HISTAMINE DIHYDROCHLORIDE	MORTALITY IN 48 HOURS	AVERAGE TIME OF DEATH AFTER HISTAMINE
		mgm. per kgm.	*per cent*	*hours*
6	Normal	650	0	
4	Normal	1,000–1,200	0	
2	Normal	1,500	0	
2	Hypophysectomised	50	0	
8	Hypophysectomised	200	12.5	
5	Hypophysectomised	500	40.0	36
21	Hypophysectomised	650	85.7	20
6	Adrenalectomised	200	33.3	
8	Adrenalectomised	500	62.5	17
11	Adrenalectomised	650	90.9	7.4

TABLE 2

Toxicity of histamine on treated hypophysectomised rats

NUMBER OF RATS	CORTICOTROPHIC EXTRACT NUMBER	DAILY DOSE	DOSE HISTAMINE DIHYDROCHLORIDE	MORTALITY	AVERAGE TIME DEATH AFTER HISTAMINE	ADRENALS, AVERAGE WEIGHT
		cc.	*mgm. per kgm.*	*per cent*	*hours*	*mgm.*
6	331	1.5	650	66	37	22.5
4	215	1	650	50	26	25.0
5	278	1	650	0		42.5
	DESOXYCORTICOSTERONE	(TOTAL DOSE)				
		mgm.				
5	Subcutaneous crystals	5-10	650	100	34	13
6	In oil	1	650	100	28.3	

Toxicity of histamine in treated adrenalectomised rats. Immediately after adrenalectomy pellets of desoxycorticosterone of from 5 to 8 mgm. in weight were implanted into the subcutaneous tissue. In one group of rats life was maintained even though they did not receive saline to drink. The other group had saline to drink in addition to the pellets. All received histamine after 12 to 14 days of treatment.

It may be seen from table 3 that desoxycorticosterone markedly raised the resistance in some animals when compared with the figures obtained for untreated adrenalectomised rats. It appeared that the animals which showed the greatest weight gain after adrenalectomy were more resistant to histamine than those which failed to gain weight normally.

Hemoglobin values after histamine in normal, hypophysectomised and adrenalectomised rats. Hemoglobin determinations have been made on many of the above animals to give some indication of the changes occurring in the blood volume. In one experiment with normal rats the absorption of the histamine was prolonged by administering it in a 2 per cent solution of zinc acetate (1). No mortality was encountered following this procedure, but the effects on hemoglobin are included in table 4. Only a few isolated observations were made on the adrenalectomised animals.

These results indicate that in normal rats the concentration of hemoglobin is maximal very shortly following the injection of histamine, usually

TABLE 3

Toxicity of histamine in treated adrenalectomised rats

NUMBER OF RATS	NaCl TO DRINK	AT OPERATION	BODY WEIGHT CHANGE	DOSE HISTAMINE DIHYDRO-CHLORIDE	MORTALITY, 48 HOURS	AVERAGE TIME DEATH AFTER HISTAMINE
		grams	*grams*	*mgm. per kgm.*	*per cent*	*hours*
3	No	78	+47	750	0	
3	No	61	+22	650	100	20
4	Yes	157	+23	650	25	24

in 30 minutes, and in some animals where determinations were made, as early as 15 minutes. Following this the hemoglobin returns gradually to normal, but with the larger dose of histamine it may still be raised after 6 hours. Values below normal usually are found at a still later period. Following a histamine dose of 1500 mgm. per kgm. a rapid fall in hemoglobin occurred following the initial rise. In the experiment where the absorption of the histamine was retarded a gradual increase in hemoglobin values continued up to 6 hours, differing markedly from the effects of a similar dose of histamine alone, but with no change in mortality. In hypophysectomised animals the changes in hemoglobin values are essentially similar to those found for normals, except that even with small doses of histamine the increased hemoglobin values usually continue for at least 6 hours. In adrenalectomised animals some extremely high values were noted.

Hemoglobin values in treated hypophysectomised rats. Hemoglobin determinations were made on the hypophysectomised rats treated with corticotrophic extracts and with desoxycorticosterone as previously described. These results may be seen in table 5. In these animals it

TABLE 4

Hemoglobin values in untreated normal, hypophysectomised and adrenalectomised rats

NUMBER OF RATS	CONDITION	DOSE HISTAMINE DIHYDROCHLORIDE	GRAMS HEMOGLOBIN PER 100 CC. C.	PERCENTAGE HEMOGLOBIN						
				c.	½ hour	1 hour	2 hours	4 hours	6 hours	24 hours
		mgm. per kgm.								
6	Normal	650	17.27(16.39) (18.76)	100	114(106) (125)	109 (98) (119)	99 (90) (108)	98 (90) (104)	97 (90) (101)	95 (88) (100)
2	Normal	650 (retarded)	14.89(14.70) (15.08)	100	108(107) (109)	107(107) (107)	108(107) (109)	114(113) (115)	116(114) (118)	94 (93) (95)
4	Normal	1000-1200	13.55(12.62) (14.30)	100	125(121) (129)	121(116) (136)	112(102) (125)	109(106) (116)	104(100) (109)	92 (87) (78)
2	Normal	1500	13.92(13.35) (14.50)	100	113(112) (114)	104(101) (107)	96 (84) (108)	98 (94) (102)	100 (97) (103)	84 (78) (90)
2	Hypophysectomised	50	17.[illegible] (18.48)	100	108(107) (109)	105(102) (108)	108(106) (110)	104 (99) (109)	104(100) (108)	92 (90) (94)
4	Hypophysectomised	200	17.09(15.83) (19.08)	100	119(118) (119)	114(112) (116)	113(108) (116)	108(103) (112)	102 (99) (105)	96 (92) (100)
10	Hypophysectomised	650	15.06(12.08) (19.66)	100	124(111) (140)	120(108) (139)	114(102) (129)	112(103) (124)	115(111) (130)	114(107) (121)
2	Adrenalectomised	500	13.93(13.74) (14.12)	100	124(120) (129)	117(112) (122)	113(110) (117)	113(109) (117)	112(105) (120)	86 (85) (87)
2	Adrenalectomised	650	13.49(13.16) (13.82)	100	148(144) (152)	142(136) (149)	133(127) (140)	122(122)		

TABLE 5

Hemoglobin values in treated hypophysectomised rats

NUMBER OF RATS	TREATED EXTRACT	DOSE HISTAMINE DIHYDROCHLORIDE	GRAMS HEMOGLOBIN PER 100 CC. C.	PERCENTAGE HEMOGLOBIN							MORTALITY
				c.	½ hour	1 hour	2 hours	4 hours	6 hours	24 hours	
		mgm./kgm.									*per cent*
6	Corticotrophic 331	650	13.16(11.25) (14.89)	100	117(108) (124)	114(108) (120)	112(105) (116)	116(111) (122)	112(105) (117)		66
4	Corticotrophic 215	650	15.37(13.35) (17.31)	100	116(106) (126)	115(108) (120)	116(113) (119)	121(120) (122)	118(105) (124)	113(111) (116)	50
5	Corticotrophic 278	650		100	114(108) (122)	109(102) (116)	103 (99) (107)	105(101) (114)	102 (98) (105)	98 (94) (101)	0
5	Desoxycorticosterone—crystals	650	14.29(12.08) (15.83)	100	121(117) (126)	119(109) (125)	117(109) (123)	116(107) (123)	114(109) (116)	117(113) (126)	100
6	Desoxycorticosterone—oil	650	15.95(13.54) (17.31)								100

may be seen that the hemoglobin values remained high up to 6 hours in the instances where little or no protection was afforded by the treatment. However, in the group of rats completely protected, the hemoglobin values, following the initial increase, returned rapidly to normal in a fashion similar to that found to occur in normal intact rats.

Effect of histamine on fluid intake and urine output. A number of the rats which were treated with histamine were maintained before and after injection under conditions so that measurements of fluid intake and output could be made. These results have been recorded in table 6. In the normal animals it may be noted that in the first hour after histamine an increase in water intake occurred. During this period the rats were obviously thirsty and lay near the water supply. After the first hour little

TABLE 6

Water intake and urine output in normal and hypophysectomised rats after histamine

NUMBER OF RATS	CONDITION	DOSE OF HISTAMINE	AVERAGE OF PRECEDING 3 DAYS	AVERAGE PER RAT—CC. IN HOURS AFTER HISTAMINE					
				½	1	2	4	6	24
		mgm./kgm.							
6	Normal	650							
		Water intake	24.8	6.4	9.4	10.4	10.4	10.9	32.1
		Urine output	7.0	0	0	0	3.0	10.2	27.0
12	Hypophysectomised	50-650							
		Water intake	17.0	1.6	2.2	2.3	2.3	2.3	9.8
		Urine output	7.6	0	0	0	0	0.1	4.3
5	Hypophysectomised (treated—ext. 278)	650							
		Water intake	29.2	2.2	2.2	2.2	2.4	2.4	13.2
		Urine output	16.4	0	0	0	1.2	1.8	9.3

more water was consumed. The urine output was negligible for the first two hours and then diuresis commenced. For the 24 hour period after histamine the water intake was always increased and the urine output markedly so when compared with the values obtained preceding the injection. Similar changes have been observed previously by Howlett and Browne (2). In the hypophysectomised animals a somewhat different picture was presented. In no case was polyuria encountered and the water intake, while increased initially, was never found to be above the control figures. Similarly, in the rats treated with corticotrophic extract 278, which afforded complete protection from histamine, the fluid changes were as those described for hypophysectomised and not for normal animals.

It was thought that the inability of the hypophysectomised rat to excrete ingested or injected water in a normal manner might be a factor in the failure of the above animals to develop polyuria after histamine. A

series of rats were therefore injected intraperitoneally with 5 per cent of their body weight of water. This was preferable to oral administration, as previous starvation was not necessary. Normal rats were found to excrete approximately 50 per cent of the injected water within 2½ hours. In 65 tests on hypophysectomised rats, however, less than 0.5 per cent of the injected water was excreted. The hypophysectomised animals treated with extract 278, as just described, also showed this failure to develop a diuresis following injected water.

Discussion. In the experiments described the toxic effects of histamine on rats have been determined after removal of the pituitary and adrenal glands and after subsequent replacement therapy. It has been found that hypophysectomy or adrenalectomy lowers the resistance of the rats so that death results from a dose of histamine which does not kill normal animals. Under the experimental conditions rats adrenalectomised from 6 to 8 days were slightly more susceptible than those tested two weeks after hypophysectomy. This observation would suggest that the atrophic adrenals of the hypophysectomised animal are still functional to some extent. This has frequently been suggested from the evidence that the hypophysectomised rat does not die of acute adrenal insufficiency. Treatment after hypophysectomy with a suitable corticotrophic extract was followed by an increase in weight of the adrenal glands, and an apparent increased resistance of the animal to histamine. These results confirm the findings reported by Perla and his associates in a series of observations. They have shown that not only cortical extract or corticotrophic extracts effectively increased the resistance of hypophysectomised rats to histamine (3, 4, 5), but also the beneficial effects of cortin in adrenalectomised rats (6).

The adrenalectomised animals which were studied were maintained on NaCl and such animals were obviously more susceptible to histamine than were normal rats. It is probable that cessation of salt treatment would have resulted in an even increased susceptibility as the general condition of the animal deteriorated. These results, therefore, confirm Perla and Sandberg (7) who state that "administration of salt to suprarenalectomised rats will raise the resistance slightly, but not to a degree comparable to that obtained with injections of suprarenal cortical hormone." They do not confirm the generalization made by Selye (8) that with salt treatment "the resistance to drugs is almost completely restored to normal" in the adrenalectomised rat. Treatment of the adrenalectomised rats by desoxycorticosterone with or without salt apparently resulted in a lowered mortality from histamine. A difference in mortality was apparently associated with the response to treatment as indicated by the weight increase of these animals. Those which gained the greatest in weight resisted successfully the effects of the histamine. On the other hand, in hypophysectomised rats desoxycorticosterone was of no protective value.

The changes in hemoglobin have been considered as an approximate index of the alterations in blood volume in the animal. Following a moderate dose of histamine the normal rat showed definite hemoconcentration which was maximal in about 30 minutes after the injection. Thereafter, dilution occurred so that the hemoglobin was normal after a few hours and, ultimately, at 24 hours, evidence of increased blood dilution was found. With larger doses of histamine the changes were similar except that the initial hemoconcentration was greater, the following dilution occurred more gradually, and by 24 hours considerable blood dilution had occurred. The hypophysectomised and adrenalectomised rats showed similar alterations but all these were increased in magnitude. The blood concentration was usually greater, especially after adrenalectomy, than that found in normal rats, and the following dilution was very gradual, so that even after 6 hours the blood was still concentrated. When hypophysectomised rats were treated with corticotrophic extract 278 the changes in hemoglobin were similar to those noted for normal rather than for hypophysectomised rats. These results indicate that the adrenal glands are intimately associated with the processes which enable blood dilution to occur following the initial hemoconcentration. In general, it may be seen that the groups of rats which showed less initial hemoconcentration and a more rapid and complete secondary blood dilution were ones which were not killed by the histamine treatment. This would suggest that the mortality might be related to the changes associated with hemoconcentration per se. However, when individual animals were considered, it was noted that animals may show relatively good blood dilution and yet die. Conversely, rats in which the blood remained concentrated sometimes survived. The latter condition was readily produced in normal rats by retarding the absorption of the histamine. Changes in blood volume, therefore, could be used as an approximate indication as to whether fatality would occur, but only in large groups of animals and not in individual cases.

The actual hemoglobin content of the blood of the rats before histamine treatment showed extreme variations. Even in the normal animals values from 12.62 to 18.76 grams per 100 cc. were encountered. This would suggest the imperativeness of obtaining hemoglobin values before and after subjecting rats to conditions simulating shock, rather than to compare the hemoglobin of one group of normal animals with that of another group after shock (9). Admittedly, the use of hemoglobin changes as an indication of changes in blood volume is open to criticism. In the experiments described, however, the changes in hemoglobin have occurred in an orderly fashion, and as such are probably a fairly true indication of changes in the blood volume. In one case where histamine in a dose of 1500 mgm. per kgm. was injected, an unusual curve for hemoglobin was obtained.

possibly related to the amount of solid injected. It is believed that the technique described entailed less chance of error than direct measurement of the "freely circulating blood" which might be collected following the cutting of the carotid artery and jugular vein, as has been advocated (10).

It was hoped that the findings which were obtained might be related to the general problem of surgical shock, although histamine while producing some changes similar to those seen with shock, has never been proven to be a factor in the etiology of that condition. The use of histamine as a chemical method for the production of a shock-like condition, however, is probably more within the limits of physiological possibilities than treatment with more drastic substances, such as formaldehyde. The mechanism of the hemoconcentration in shock has been discussed by Moon (11), and is supposedly associated with an increased permeability of damaged capillaries allowing the escape of plasma from the blood stream. The rapidity with which both the concentration and dilution of the blood was found to occur in the normal rats suggests that the alteration in capillary permeability was very brief: damage in a structural sense to the capillaries could hardly have taken place. In hypophysectomised animals, although the duration of the hemoconcentration was more prolonged, the initial values were only slightly increased—were capillary damage the explanation of the prolonged blood concentration one might expect much higher initial levels. From these results it might be suggested that some change in the blood itself or in the tissues with a resulting transfer of plasma would seem a more probable explanation than a primary local damaging action on the capillaries.

The restoration of the adrenal glands of the hypophysectomised rat to normal and the associated increased resistance to histamine is of interest since adrenal extracts have been used in shock therapy. Beneficial results of cortin treatment have now been reported by a number of workers both in experimental animals and in humans. A recent review of these reports is contained in the article by Weil, Rose and Browne (12). The effects of desoxycorticosterone alone are more difficult to evaluate as there are a number of contradictory publications on its value (13). The findings of Weil, Rose and Browne (12), and of Selye and Dosne (14) suggest that desoxycorticosterone alone has little value in preventing mortality following experimentally produced shock. Corticosterone, on the other hand, seemed much more effective. In a recent publication Swingle, Hays, Remington, Collings and Parkins (15) have found that desoxycorticosterone would effectively prevent mortality following muscle trauma in adrenalectomised dogs, but had no protective value following trauma of the gastrointestinal tract. This suggests a possible difference in etiology for those types of shock (or at least a quantitative difference) and may help to explain the discrepancies in previous reports.

SUMMARY

The toxicity of histamine has been studied in normal, adrenalectomised and hypophysectomised rats. Similarly, hypophysectomised rats treated with corticotrophic extract or with desoxycorticosterone acetate and adrenalectomised rats treated with desoxycorticosterone acetate have been tested. Treatment of hypophysectomised rats with corticotrophic hormone greatly increased their previously lowered resistance to histamine but desoxycorticosterone had no effect. Adrenalectomised rats maintained on oral saline showed a low tolerance to histamine, but this could be increased by desoxycorticosterone treatment.

Repeated hemoglobin determinations were made on many of the rats studied as an indication of changes in blood volume. Normal animals showed hemoconcentration to be maximal 30 minutes after the histamine injection. Thereafter, blood dilution took place rapidly, so that the normal values were obtained by 6 hours. Further blood dilution was seen at 24 hours. Increasing the dose of histamine led to a slightly increased degree of concentration, and a more gradual hemodilution. These changes were all exaggerated in hypophysectomised and adrenalectomised animals. When the former rats were treated with a suitable corticotrophic extract affording protection, the hemoglobin changes found resembled those described for normal rats.

Polydipsia was observed during the first hour only after histamine, and in normal animals polyuria always occurred in the first 24 hours. In hypophysectomised animals polyuria was never observed. The latter observation may be related to the inability of the hypophysectomised rat to develop polyuria following the ingestion or injection of water. Corticotrophic hormone did not restore this derangement in water excretion.

This research has been conducted through the support of the National Research Council of Canada. We wish to thank Mr. C. Larsen for technical assistance in these experiments. The desoxycorticosterone acetate was generously supplied by Dr. E. C. Schwenk of the Schering Corporation, Bloomfield, N. J.

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THE INEFFECTIVENESS OF VAGAL STIMULATION ON VENTRICULAR FIBRILLATION IN DOGS[1]

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Scattered reports can be found which suggest that stimulation of a vagus may abolish or modify the character of ventricular fibrillation. The most systematic study was that of Garrey (1), who concluded that "in a certain, though small, percentage of dogs stimulation of the vagus nerve will alter the character of or even stop ventricular fibrillation." The fact is stressed, however, that some aberrant condition of the myocardium must exist, in order to achieve such effects.

In view of a possible practical value of the method in cardiac resuscitation, the efficacy of the procedure has been tested over a period of 17 years in this laboratory and, following the general suggestions of Garrey, serious attempts have been made to discover conditions which might favor such recovery. A review of protocols of experiments from May 29, 1923 to the present day discloses that one or several attempts to restore coördinated beats through vagus stimulation were made in 78 dogs. These and many unrecorded trials were all negative. Hence, the briefest possible summary is indicated.

Dogs were anesthetized in various ways, among them by chloretone, sodium barbital, amytal, dial and ether, usually, but not always, preceded by 2 cc. 2 per cent solution morphine sulfate. In most of the experiments both vagi were excited in turn by the strongest feasible induction shocks; in two experiments A-C currents ranging from 30 to 1000 cycles per second were used. In many experiments both vagus nerves were excited simultaneously, hoping to improve the quantal value of impulses.

Our conclusion that the vagus nerve is incapable of influencing ventricular fibrillation is based on experiments empirically grouped as follows:

1. A group of experiments, carried out in conjunction with Doctors Bell, Theisen, Shaw and Paine (2) in 1929–30, on 18 dogs in which fibrillation was induced by faradic stimulation of the right ventricle. In each of these, the right or left vagus nerves were stimulated $\frac{1}{2}$ to 6 minutes after

[1] The continuance of these investigations was made possible by a grant from the John and Mary R. Markle Foundation.

induction of fibrillation. The observations were all made at the beginning of an experiment. The effects were consistently negative. All animals died of fibrillation.

2. A group of experiments on 35 dogs in which fibrillation was due to, or associated with, various acts or procedures. Thus, fibrillation followed an induced premature contraction, insertion of an optical manometer through the myocardium, excessive hyperthermia, severe hypercapnia, use of KCl, quinidine, infusion of digitalis, strophanthin, quinidine, thiamine, lactic acid, etc. In all except four of these, fibrillation occurred toward the end of experiments which were 3 to 5 hours in duration. The heart was in poor condition dynamically, but conduction disturbances were present only in the experiments involving use of KCl, quinidine, digitalis and strophanthin. In no instance was revival accomplished.

3. A group of experiments on 12 dogs performed in association with Doctors Orias, Cotton, Tennant and Green, in which fibrillation followed after continued coronary occlusion or upon release of a temporarily occluded coronary vessel. In these experiments, fibrillation occurred at various intervals after the beginning of an experiment. By this time we were beginning to employ the countershock method of Hooker et al., and in 5 dogs this revived the ventricles when vagus stimulation applied $\frac{1}{2}$ to 2 minutes after onset of fibrillation had been without effect. In 6 dogs no revival occurred; in the other countershock was not tried.

4. A group of experiments on 8 dogs carried out in association with H. Wiggers in which fibrillation occurred during coronary occlusion (ramus descendens). With the recognition that fibrillation evolutes through four stages (2), attempts were made to start the vagus stimulation during the undulatory, convulsive, tremulous and atonic phases, i.e., immediately after its induction until 4 minutes thereafter. Since such fibrillation recurred repeatedly during these experiments and could be abrogated successfully by use of countershock in the majority, the effect of vagal excitation was tried at different times of a protracted experiment, in one instance 9 times. Again the results were negative.

5. A group of experiments on 5 dogs carried out during the past year with Doctor Wégria in which fibrillation was produced by a single shock during the vulnerable phase. By use of the method of serial defibrillation (3) the ventricles were revived three to five times, when previous stimulation of both vagi proved ineffective.

Effect on the character of the fibrillation. Owing to the continual changes in the appearance of the fibrillary movements or of electrical and myographic recordings, the conclusion that visual or recorded changes observed are due to vagal stimulation must be guardedly drawn. The only changes which would be convincing to us are a reversal of tremulous to coarser or undulatory types of contraction such as follow a series of weak A-C shocks

(3). However, changes in this direction have never been witnessed or recorded. It is our impression that the course of the evoluting changes during fibrillation is not changed in the least by vagus stimulation. We have no explanation to offer as to the reason for our unfavorable results and the more favorable reports by others.

SUMMARY AND CONCLUSIONS

In numerous trials on 78 dogs in which ventricular fibrillation was induced by various means and at various times during the course of an experiment, stimulation of the vagus nerves by strong faradic shocks and at different times after onset of fibrillation never restored normal coördinated beats nor produced convincing changes in the usual trend of the fibrillating process. Two corollaries follow: *1*, the method is obviously without value as a resuscitating agent; and *2*, crucial proof that the vagus has any effect on the fibrillating or normally contracting dog's ventricle still remains to be produced.

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SALIVATION IN RESPONSE TO LOCALIZED STIMULATION OF THE MEDULLA

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The position of the salivary nuclei and the intramedullary course of their efferents have not been precisely determined, and the literature on the subject is controversial. Claude Bernard (1856) observed a submaxillary flow in the dog upon puncturing the floor of the fourth ventricle slightly in front of the diabetic center, a little behind the origin of the trigeminal. He gives no information as to whether this flow was ipsilateral or bilateral. Loeb (1870) was able to secure predominantly ipsilateral parotid as well as bilateral submaxillary salivation also by damaging the floor of the fourth ventricle in dogs. Grützner (1873) stimulated the medulla of dogs electrically and obtained a copious predominantly ipsilateral submaxillary secretion. Beck (1898) destroyed various parts of the medulla in dogs and found that reflex submaxillary salivation was unimpaired so long as the region of the facial nucleus remained undamaged. Köster (1900), on the basis of rather scanty clinical evidence, believed the salivary centers lay caudal to the facial nucleus.

Kohnstamm (1902a, 1902b, 1903, 1907), using the method of chromatolysis in dogs, described a submaxillary center with crossed and uncrossed efferents lying widely distributed in the reticular formation above the facial and trigeminal motor nuclei, and a parotid center, likewise with crossed and uncrossed efferents, lying between the inferior olive and the nucleus ambiguus. Bechterew (1908) and Solomowicz (1908) extirpated the submaxillary gland in dogs; their results, in general, agreed with those of Kohnstamm.

After cutting the chorda tympani in dogs, Yagita and Hayama (1909) observed degenerated cells of the visceral motor type lying in the reticular formation above the posterior third of the facial nucleus, predominantly on the operated side. After sectioning the tympanic nerve, they concluded that the parotid center was a direct caudal continuation of the submaxillary center. Later Yagita (1909) repeated the latter operation with more

[1] This work was carried out in the laboratory of Dr. R. S. Morison, to whom I am extremely grateful for advice during the experiments and the writing of this paper. I also want to thank Mr. John Galt for his assistance at the operative procedures.

care, and described a parotid nucleus most conspicuous between the caudal end of the facial nucleus and the cranial end of the nucleus ambiguus, ventromedial to the spinal vestibular nucleus. Again the degenerated cells lay mostly on the operated side.

By unipolar faradic stimulation of the surface of the medulla in cats, Miller (1913) was able to locate two points, one giving rise to submaxillary, the other to parotid secretion. Both results were ipsilateral, and no salivation was obtained from stimulation in the midline.

In brief, several investigations, predominantly carried out on the dog, have shown that in the medulla there are a submaxillary and a parotid secretory nucleus. There is no general agreement as to their exact position, nor on the question of whether their efferents are ipsilateral or bilateral. The present paper is a report of the further localization of the salivary centers in the cat by means of exploration of the medulla with stimulating electrodes.

Method. Cats anesthetized with dial (Ciba, 0.7 cc. per kgm. intraperitoneally) were used. Fine glass cannulas were inserted into the submaxillary and parotid ducts. Errors due to differences in the calibers of the cannulas were eliminated by having an observer record the number of drops of saliva falling from each cannula after an intravenous injection of acetylcholine.

The occiput was exposed by dissection of the overlying muscles, the atlanto-occipital membrane reflected, and the bone removed with rongeurs until sufficient exposure of the medulla was obtained. The cerebellum was left in place, the electrode being inserted through it when the occasion arose. In some of the cats the cervical sympathetics were cut bilaterally to insure that only the parasympathetic centers were involved. This procedure appeared to make no significant difference in the results. In several experiments blood pressure was recorded with a mercury manometer connected to a femoral artery.

For exploration a concentric bipolar electrode was used, mounted in a metal frame in such a way that its position in space could be read on three scales. This instrument has been described elsewhere as adapted for use as a modified Horsley-Clarke apparatus (Morison, Dempsey and Morison, 1941). Current was supplied by a Harvard inductorium with 6 volts in the primary circuit and the secondary at a distance of 12 cm. Whenever salivation was obtained an observer recorded the number of drops, the side, the readings on the scales at that point, and the reading on the vertical scale when the electrode just touched the surface of the medulla or cerebellum (to avoid later errors due to shrinkage of the fixed brain). At the conclusion of each experiment the medulla, pons and cerebellum were removed intact and fixed in formalin for microscopic examination of serial sections after Nissl staining.

RESULTS. A. *Areas producing salivation.* Stimulation of the medulla at levels between the principal nucleus of the fifth nerve anteriorly and the rostral level of the nucleus ambiguus posteriorly gave rise to activation of the salivary glands. In the majority of positions (fig. 1, areas 1 and 3), some secretion was produced in both the submaxillary and parotid glands homolateral to the side stimulated.

Areas which gave rise to activity of the parotid only were found scattered through the reticular substance especially in regions medial, dorsal and posterior to the facial nucleus and anterior to the ambiguus (fig.

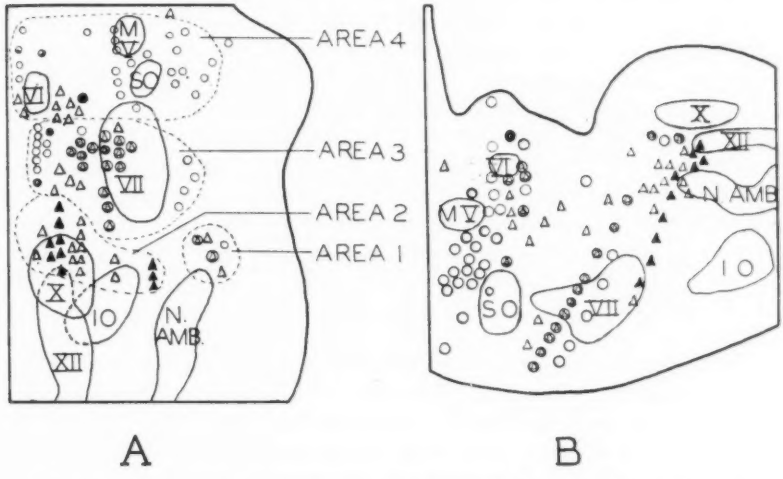

Fig. 1. Diagrammatic reconstruction of the medulla of the cat (after Winkler and Potter), showing the location of points where stimulation yielded salivation. A, dorsal view; B, sagittal projection. Open triangles indicate ipsilateral parotid salivation; solid triangles, bilateral parotid salivation; hollow circles, ipsilateral submaxillary salivation; solid circles, bilateral submaxillary salivation (1 instance). Roman numerals refer to nuclei. *MV*, motor V; *N.AMB.*, nucleus ambiguus; *SO*, superior olive; *IO*, inferior olive. Areas outlined by stippling are explained in text.

1, area 2). Points rostral to the facial nucleus yielded predominantly submaxillary effects (fig. 1, area 4).

Bilateral effects were occasionally encountered. In one instance both submaxillary glands were activated by stimulation of a point near the lateral border of the genu of the facial nerve. Bilateral parotid effects were recorded more posteriorly.

B. *Associated striated muscle movements.* In confirmation of the anatomical identification of the points mentioned above, the most marked submaxillary salivation was accompanied by movement of the facial

musculature. Movements of the pharynx and larynx were frequent accompaniments of maximal parotid effects.

C. *Blood pressure changes.* As was to be expected from the proximity of the regions stimulated in these experiments to the location of the vasomotor "center," blood pressure changes were frequently produced. Salivation was, however, not significantly affected, similar results being encountered either in the presence or absence of the alterations in vascular tension.

Discussion. In any stimulations of the central nervous system such as those reported here, the question arises as to whether fibres or nuclear structures are being activated. Presumably both types of elements were involved in the present study and account, at least in part, for the results obtained. Simultaneous activation of the submaxillary and parotid glands of one side and the frequent bilateral effects observed suggest the involvement of afferents, especially taste fibres from the glossopharyngeal or nervus intermedius. Thus in figure 1, area 1 presumably represents taste fibres or cells associated with the tractus solitarius; and area 3 may be thought of as containing association fibres of one sort or another running to the predominantly submaxillary nucleus anteriorly and the parotid nucleus dorsally and posteriorly. Crossed efferent fibres from the salivary nuclei have been mentioned by various observers, as pointed out in the introduction to this paper. The *a priori* more likely possibility of crossed afferents renders it impossible to decide the question of crossed efferents on the basis of the present experiments.

The finding of predominantly submaxillary points in the position indicated as area 4 (fig. 1) and including the rostral parts of area 3 corresponds well with the position of the nucleus as outlined by retrograde degeneration after cutting the chorda tympani in the dog (Yagita and Hayama, 1909) and with the efferents in the facial nerve. Area 2 and some of area 3 which gave strong parotid effects lie close to regions similarly indicated by Yagita (1909) for the parotid. Presumably the greater concentration of active elements supplied by the grouping of afferents, efferents and cells in nuclear regions results in the relative predominance of their responses.

SUMMARY

By electrical exploration of the medulla in cats, it was found that stimulation of various points yielded salivation. These points lay mostly in the reticular formation and in the region of the intramedullary course of the facial or glossopharyngeal nerves.

Points which gave predominantly submaxillary salivation, with the exception of those associated with the facial efferents, lay mostly rostral

and medial to the middle third of the facial nucleus. These points are regarded as representing the submaxillary center.

Points primarily concerned with parotid salivation were concentrated caudal to and above the submaxillary center, and medial to and above the cranial end of the nucleus ambiguus.

Both ipsilateral and bilateral salivation were obtained from unilateral stimulation, with or without accompanying blood pressure rises.

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RESPIRATORY MODIFICATION OF THE CARDIAC OUTPUT

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Alterations of intrathoracic pressure associated with the different phases of respiration are considered to be of importance in facilitating the return of blood to the thorax and, consequently, respiratory variations of cardiac output. Many studies have been made of direct cardiac volume changes with open chest and artificial respiration, but observations with intact thorax and normal respiration have been rare. The literature up to 1921 has been critically reviewed by Wiggers (1921), and references in the present report will be mainly confined to more recent work.

It has been generally assumed that increased depth and rate of respiration increases the "aspiratory" effect of the thorax and hence the inflow of blood to the right heart (Burton-Opitz, 1902, 1914; Hooker, 1921; Wiggers, 1921; Visscher, Rupp and Scott, 1924; Heinbecker, 1927). Thus, by increasing the inflow of blood during inspiration or during exercise, there is necessarily an augmented output of the right (Heinbecker, 1927) or both ventricles (White and Moore, 1925). There was no direct experimental evidence in support of these views until Eyster and Hicks (1933) made direct cardiac output determinations with a closed chest. They found inspiration to be associated with a greater diastolic size than expiration, but a decreased stroke volume. These results are in apparent contradiction to the Starling principle.

1. *Ventricular volume changes.* Medium-sized dogs anesthetized with sodium barbital were used. With artificial respiration supplied by means of interrupted blasts, the fifth rib on the left was resected and a metal truncated cone window sutured into this space. Covering the exposed lumen of the cone with membrane rubber, which could be rapidly removed, made possible the sudden production of pneumothorax. The fourth and fifth ribs on the right were resected, the pericardium removed and the two ventricles enclosed in a volume recording glass oncometer which was a modification (in shape) of that first described by Wiggers and Katz (1922). Care was taken to insure a proper fit of the rubber membrane of the oncometer at the A-V groove, to avoid both leakage and constriction. Before suturing the chest closed, a small metal trocar was inserted through a stab wound in the chest wall, supplying means for aspiration of air from the thorax as well as regulating and recording intrathoracic pressure.

The method of recording was adapted to the stationary optical manometer devised by Hamilton, Brewer and Brotman (1934). The oncometer was attached to a manometer equipped with a rubber, rather than a metal, membrane (Frank, 1903). At the close of each experiment, after cessation of the heart beat, the oncometer was calibrated by injecting into the oncometer-manometer system, or withdrawing from it, known volumes of air. No attempt was made to determine absolute ventricular volumes. Carotid blood pressure was recorded with a manometer equipped with a silver membrane of sufficient natural frequency and sensitivity for accurate pressure pulse recordings. Intrathoracic pressure and pneumographic

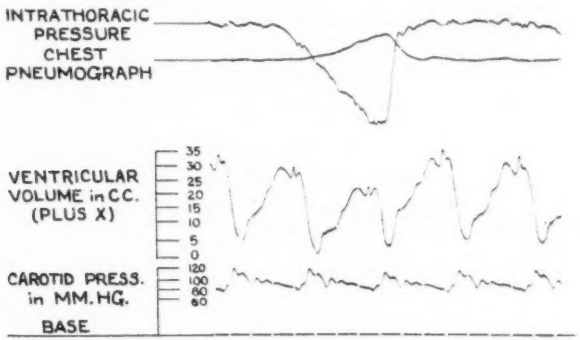

Fig. 1. Simultaneous curves of intrathoracic pressure, chest pneumograph, ventricular volume and carotid blood pressure. Downstroke of ventricular volume curves is systole. X is absolute ventricular volumes, which were not measured. Downstroke of intrathoracic curve is inspiration. Values listed are from single cardiac cycles at the end of inspiration and completion of expiration. Time = 0.1 sec.

	Inspiration	Expiration
Diastolic volume	X + 18 cc.	X + 29 cc.
Stroke volume	17 cc.	25 cc.
Systolic pressure	113 mm. Hg.	119 mm. Hg.
Diastolic pressure	76 mm. Hg.	82 mm. Hg.

tracings were also recorded with rubber, rather than metal, membranes. The former was calibrated against a column of water and, in all experiments, so regulated as to be within the normal limits of 3 cm. (negative) of water in expiration to 7 cm. (negative) of water in inspiration.

In figure 1, it will be noted that the relative diastolic size was 18 cc. in inspiration, and 29 cc. in expiration, an increase of 61 per cent. Stroke volume likewise increased in expiration from 17 cc. to 25 cc. (47 per cent). Systolic blood pressure was 113 mm. Hg in inspiration, 119 mm. Hg in expiration, an increase of 5.3 per cent. Diastolic blood pressure increased 8 per cent in expiration from 76 mm. Hg to 82 mm. Hg. Respiratory

variations of systemic arterial blood pressure of similar magnitude have been observed in normal unanesthetized animals, thus lending evidence to the supposition that the arterial pressure and stroke volume changes are real and not the result of placing the ventricles in an oncometer. Furthermore Henderson and Barringer (1913) observed the respiratory changes in intrathoracic pressure exerted a negligible effect upon the thick walled ventricles. The systemic arterial pressure variations are therefore due in part to the respiratory variations in stroke volume.

Figure 2, taken 6 breaths after figure 1, illustrates the minimal changes occurring during pneumothorax. The relative diastolic volume increased

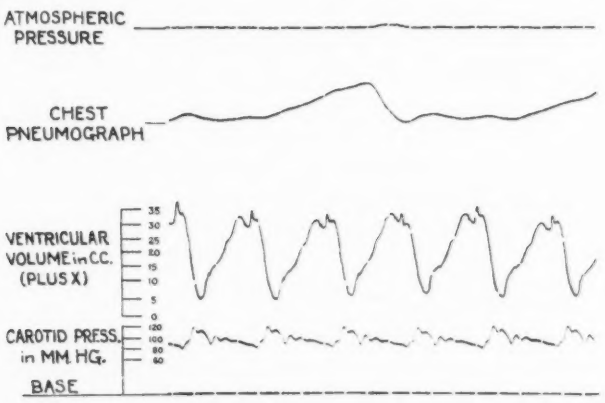

Fig. 2. Record similar to that of figure 1, but taken during pneumothorax. Intrathoracic pressure curve now registers atmospheric pressure. Time = 0.1 sec.

	Inspiration	Expiration
Diastolic volume	X + 28 cc.	X + 30 cc.
Stroke volume	22 cc.	23 cc.
Systolic pressure	121 mm. Hg.	120 mm. Hg.
Diastolic pressure	84 mm. Hg.	84 mm. Hg.

very little in expiration (from 28 cc. to 30 cc.); stroke volume remained almost constant (changing from 22 cc. to 23 cc.); and systolic and diastolic blood pressures scarcely varied throughout the respiratory cycle. These changes are insignificant when compared to those occurring with intact thorax. Although the intrathoracic curve registered atmospheric pressure after the production of pneumothorax, the latter may have been incomplete, allowing for some lung expansion, however small. This may conceivably account for the slight changes that did occur. Movements of the oncometer, incidental to respiratory movements of the diaphragm and chest wall, were necessarily the same both before and during pneumothorax, and therefore cannot be held accountable for volume and pressure variations.

Figure 3 is a record similar to that in figure 1, showing the cyclic fluctuations throughout nearly two respiratory movements. The changes are of the same order as those in figure 1. Figure 4 is a record taken during the

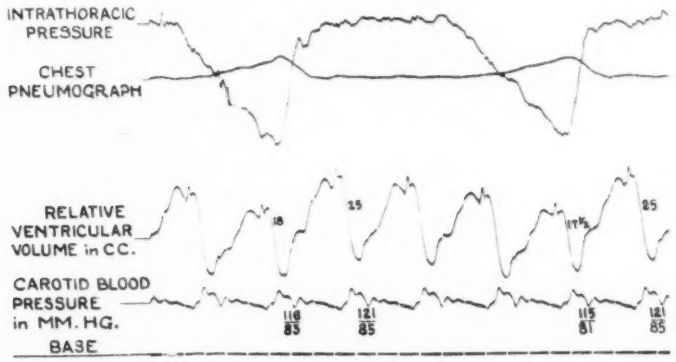

Fig. 3. Cyclic ventricular volume and arterial pressure changes with respiration. Stroke volume values are labeled to the right of, and systemic blood pressure beneath their corresponding contours for the single cardiac cycles at the end of inspiration and the completion of expiration. Time = 0.1 sec.

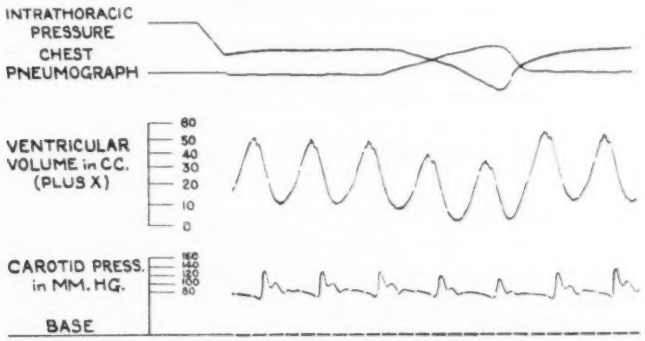

Fig. 4. Ventricular volume and arterial pressure changes with respiration. Both vagi sectioned. Time = 0.1 sec.

	Inspiration	Expiration
Diastolic volume	X + 31 cc.	X + 53 cc.
Stroke volume	28 cc.	39 cc.
Systolic pressure	114 mm. Hg.	132 mm. Hg.
Diastolic pressure	65 mm. Hg.	74 mm. Hg.

exaggerated breathing movements following double vagotomy. All effects were accentuated. Diastolic size in the single cardiac cycle at the completion of expiration showed an increase over the single cycle at the

end of inspiration of 71 per cent (31 cc. to 53 cc.); stroke volume, an increase of 40 per cent (28 cc. to 39 cc.); systolic pressure, an increase of 15.8 per cent (114 mm. Hg to 132 mm. Hg); and diastolic pressure, an increase of 13.8 per cent (65 mm. Hg to 74 mm. Hg). Time intervals (0.1 sec.) recorded on base line, indicate no appreciable change in the heart rate during the respiratory phases in any of the ventricular volume experiments.

Opposed to these findings, Eyster and Hicks (1934) observed the diastolic volume of the two ventricles to increase during inspiration, attributing this enlargement to the aspiratory effect of the thorax facilitating greater ventricular filling volumes. They nevertheless found inspiration to be associated with a decreased stroke volume. Our ventricular volume records, on the other hand, show that in inspiration both diastolic and stroke volumes decrease together, as might be expected from the law of Starling. The fall in systemic arterial pressure during inspiration has been observed by Henderson and Barringer (1913), Burton-Opitz (1921), Heinbecker (1927) and Johnson, Hamilton, Katz and Weinstein (1937). Hamilton, Woodbury and Harper (1936) attributed the fall in pressure to superimposition of the decreased intrathoracic pressure upon the arteries of the thorax. The decreased stroke volume must of necessity be an additional factor for in all of our findings arterial blood pressure invariably fell to a greater extent than intrathoracic pressure.

2. *Auricular volume changes.* No satisfactory means for direct measurements of mammalian auricular volume have been devised. In an effort to discover any fluctuations that might occur during the respiratory cycle, motion pictures[1] were taken of each auricle with the thorax closed. Plate glass windows were sealed into either side of the chest wall at the level of the two auricles of large dogs anesthetized with sodium barbital and with appropriate artificial respiration. Normal respiration was then reinstituted and high speed motion pictures taken of each auricle. Respiratory indicators were included in the field to insure accurate selection of frames at the height of both inspiration and expiration, and when the auricles were in complete diastole. Careful counts of the number of frames included in each cardiac cycle disclosed no significant change in heart rate during the respiratory phases.

Figure 5 shows the right auricle greatly dilated in inspiration, but much smaller in expiration. Figure 6, on the other hand, shows the left auricle somewhat larger in expiration. The changes of the left auricle were much less marked and on some occasions not perceptible, but it was never observed to undergo the marked filling in inspiration, nor the rapid collapse in expiration seen in the right auricle. Assuming auricular volume at any

[1] Taken by Dr. B. M. Hair.

given time to be the sum of two factors, i.e., the amount of blood flowing into the auricle plus the stretch imposed upon the thin auricular walls by the negative intrathoracic pressure, the changes in auricular size are necessarily less marked in the left auricle. Both are subjected to the same stretch, but decreased intrathoracic pressure during inspiration also draws blood from outside the thorax into the right auricle (Visscher, Rupp and Scott, 1924). Hence the two factors sum. At the same time, blood is withheld from the left auricle in the lung bed (Heinbecker, 1927; Trimby and Nicholson, 1940) so that the two factors act oppositely here, tending to minimize any volume change that might occur.

The decreased diastolic and stroke volumes during inspiration is evidence that blood is being withheld from one or both ventricles. It is conceivable that the volume of each auricle might be a rough measure of the amount

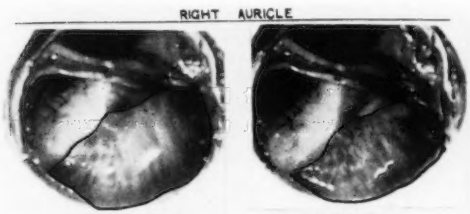

Fig. 5

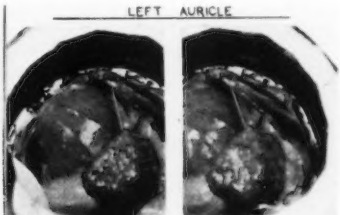

Fig. 6

Fig. 5. Photographs selected from motion picture frames showing the right auricle (outlined) in complete diastole at the height of inspiration and at the beginning of expiration.

Fig. 6. Photographs selected from motion picture frames showing the left auricle in complete diastole at the height of inspiration and at the beginning of expiration.

of blood entering or approaching its respective ventricle at any one time. These phasic changes in auricular size may then be taken to indicate one or more of the following: 1. During inspiration, blood is withheld from the left heart, accounting in part for the decreased volumes (diastolic and stroke), as well as for the fall in systemic arterial pressure. This is presumably the result of an increased storage of blood in the lung vessels. 2. At the same time, blood is aspirated into the great veins of the thorax and into the right auricle. Blood may be withheld from the right ventricle by the increased capacity of these structures as a result of a greater stretch imposed upon them than upon the thicker walled right ventricle (Henderson and Barringer, 1913). Under these conditions, the right ventricle would combine with the left in producing a decreased ventricular diastolic size and stroke volume. During expiration, blood would be squeezed from the lung bed into the left heart and from the right auricle and vena cava into

the right ventricle. The net effect would be an increase in diastolic size and, in accordance with the Starling principle, an increase in stroke volume of both ventricles. This seems the most likely explanation. 3. On the other hand, if the increased right auricular size in inspiration indicates greater right ventricular filling, the total decrease in ventricular diastolic size in this phase of respiration must be attributed wholly to incomplete filling of the left ventricle as a result of storage in the lung bed. The latter must, in this circumstance, accommodate a preponderance of blood over that aspirated into the right heart during inspiration. The total decrease in stroke volume may likewise be the result of decreased left ventricular filling volumes, since Henderson (1914) demonstrated the greater effect of filling pressures in the response of this ventricle than that of the right. The reverse would be the case in expiration, where blood squeezed from

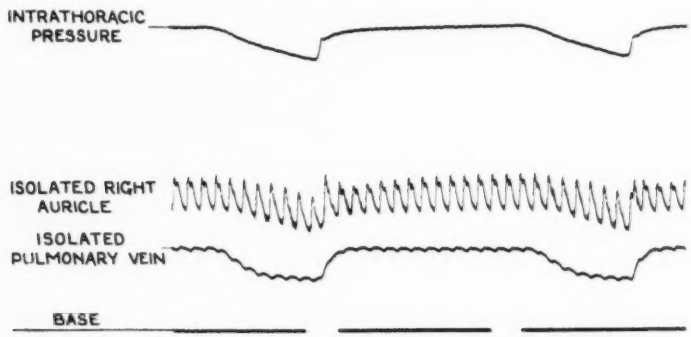

Fig. 7. Simultaneous recorded curves of intrathoracic pressure, and right auricle and pulmonary vein isolated from the circulation. Time = 5.0 sec.

the lung field alone accounts for the increased diastolic size and stroke volume.

3. *Capacity changes in the right auricle and pulmonary vessels.* Figure 7 represents an effort to demonstrate capacity changes in the pulmonary circuit and right auricle during the respiratory phases. The fourth left rib of medium-sized dogs with sodium barbital anesthesia was resected. Artificial respiration was maintained as described elsewhere. The right auricle was ligated at its junction with the vena cava. The middle lobe of the right lung was ligated at its hilus, leaving only the bronchus patent. Thus both structures were isolated from the circulation, yet left free to expand and collapse with changes in intrathoracic pressure. Glass cannulae, filled with citrate, leading to manometers covered with thin rubber, were tied into the tip of the right auricle and into a pulmonary vein at the periphery of the isolated lung lobe. In addition, both manom-

eters were connected with citrate-filled burettes outside the body. The chest was then sutured closed and normal respiration reinstituted. The curves are actually pressure changes, showing a pressure decrease in inspiration. They are reflections of capacity changes, however, as demonstrated by the fact that fluid in burettes connected with each manometer outside the chest, was drawn into the auricle and pulmonary bed with inspiration, and forced out in expiration. These findings are in confirmation with the work of many authors who have found an increased pulmonary capacity in inspiration as a result of stretch imposed upon the lung vessels (Burton-Opitz, 1921; Heinbecker, 1927; Trimby and Nicholson, 1940). Johnson, Hamilton, Katz and Weinstein (1937) also demonstrated a low elasticity coefficient in the pulmonary circuit, permitting it to store large quantities of blood with very moderate pressure increases.

SUMMARY AND CONCLUSIONS

1. In dogs under sodium barbital anesthesia in which direct cardiac volume changes of the two ventricles were measured by a cardiometer during normal breathing, there occurred during inspiration a diminished diastolic size and stroke volume.

2. Systemic arterial pressure also decreased during inspiration, mainly the result of the diminished stroke volume, since arterial pressure invariably decreased to a greater extent than intrathoracic pressure.

3. During the slow deep respiratory movements following double vagotomy, these effects were exaggerated.

4. Motion pictures were taken of the auricles during normal respiration through plate glass windows sealed into either side of the chest wall. The right auricle became larger in inspiration and smaller in expiration. The left auricle usually was observed to undergo opposite changes, but the changes were never so marked as those seen in the right auricle.

5. The decreased size of the left auricle during inspiration indicates that blood is withheld from the left heart during this phase of respiration. It is probable that the increased right auricular size observed in the inspiratory phase indicates that blood is also withheld from the right ventricle as a result of storage in the thin-walled structures on the approach to the right ventricle. Both ventricles must therefore undergo incomplete filling and decreased stroke volumes during inspiration. If, as seems less likely, the greater volume of the right auricle during inspiration indicates greater right ventricular filling, the diminished ventricular volumes must be attributed wholly to incomplete filling of the left ventricle.

6. The right auricle and middle lobe of the right lung were isolated from the circulation, yet left free to expand and collapse with changes in intrathoracic pressure. During inspiration the capacity of both structures increased, indicating a greater storage of blood in both the pulmonary circuit and right auricle during the inspiratory phase.

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COMPARISON OF THE VULNERABLE PERIODS AND FIBRILLATION THRESHOLDS OF NORMAL AND IDIOVENTRICULAR BEATS[1]

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In previous papers (1, 2) it was shown that ventricular fibrillation can be induced by application of a single induction, condenser, or brief D.C. shock during the last 0.04 to 0.06 second of systole, or the early 0.02 second of diastole. This was called the vulnerable period. In subsequent communications (2, 3), experiments were analyzed which indicated that the fibrillating potency of more prolonged direct and alternating currents depends less on the duration and strength of current than on the coincidence of an effective change in its intensity with a vulnerable period of a normal or premature beat. Finally, we suggested (4) that the spontaneous fibrillation following coronary occlusion can be explained by a release of two successive spontaneous stimuli, the first eliciting a premature contraction and the second inducing fibrillation because it falls during its vulnerable phase.

In such interpretations two assumptions were occasionally required in order to fit all cases of fibrillation into the theory that fibrillation can only be induced through incidence of an effective stimulus during the vulnerable period, viz., 1, that the major portion of the descending limb of small premature systoles is vulnerable, and 2, that the fibrillation threshold is reduced in such beats.

This paper concerns itself with a report of experiments designed to test these assumptions.

PROCEDURES. The obvious method for studying the problem was to induce premature systoles of various sizes and to apply a second strong shock at various moments of such beats. Although simple in principle, three methods needed to be used in order to cover all contingencies:

1. A weak condenser shock was applied to the left ventricle during

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[2] Fellow of the Belgian-American Educational Foundation.

[3] Porter Fellow of the Amer. Physiol. Soc.

every sixth natural beat. By keeping this shock ever so slightly out of phase with the natural beat, the ventricle was excited progressively later (or earlier) in diastole, thus yielding beats of different amplitude and form. A definite interval after such a condenser shock, a brief D.C. shock (0.01–0.02 sec.) of varying intensity was applied. This interval was changed after each set of observations.

2. The heart was driven by repeated weak condenser shocks which were applied first to the right auricle and then to the left ventricle. In each case, a brief D.C. shock of increasing strength was applied to the left ventricle during every sixth beat, a definite interval after the con-

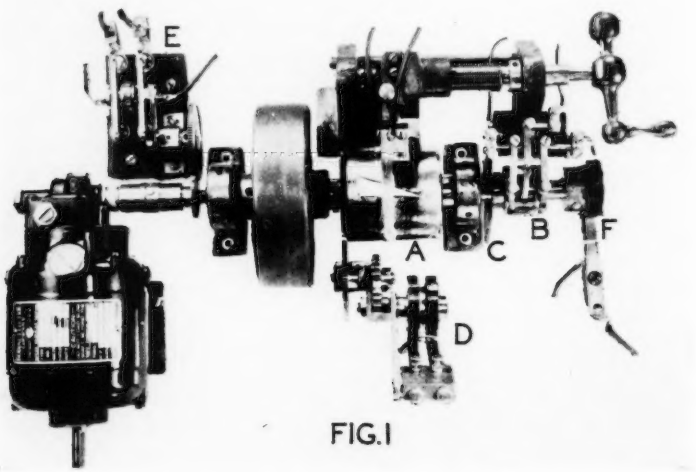


Fig. 1. Diagram of stimulator for introducing three different shocks at definitely related intervals. Discussion in text.

denser shock. This interval was also changed after each set of observations.

3. In order to compare slow beats of atrial and ventricular origin, the sinus node was first clamped, or the heart slowed by application of a 1:2000 solution of prostigmine bromide to the posterior surface of the right auricle. The heart was driven at a slow rate by weak break induction shocks applied to the right auricle. During every sixth beat, the left ventricle was excited by a weak condenser shock which automatically fell progressively later in diastole. This was followed, after a set interval, by a brief D.C. shock which fell at various moments of the induced premature beat.

A photograph of the apparatus by which as many as three such interrelated stimuli could be applied is shown in figure 1. The D.C. shock was produced and graded in intensity as described in a previous communication (2).[3] The duration of the shock was regulated by the opening of a key by the slotted cylinder *A*. The condenser shock was applied through a rotating commutator, *B*, similar to that described by A. V. Hill (5). Since this was on a common shaft with cylinder *A*, the degree of precedence of this shock could be controlled by rotating the commutator on the shaft. A calibrated index scale, *C*, facilitated such setting. In addition, a set of toothed wheels operating the primary and short-circuiting keys, *D*, of an inductorium was geared to the main shaft at a ratio of 176:175, thereby providing an arrangement by which the induction shocks were applied 6 times as often as the condenser or D.C. shocks, preceding them by a gradually decreasing interval (ca. 10 msec.).

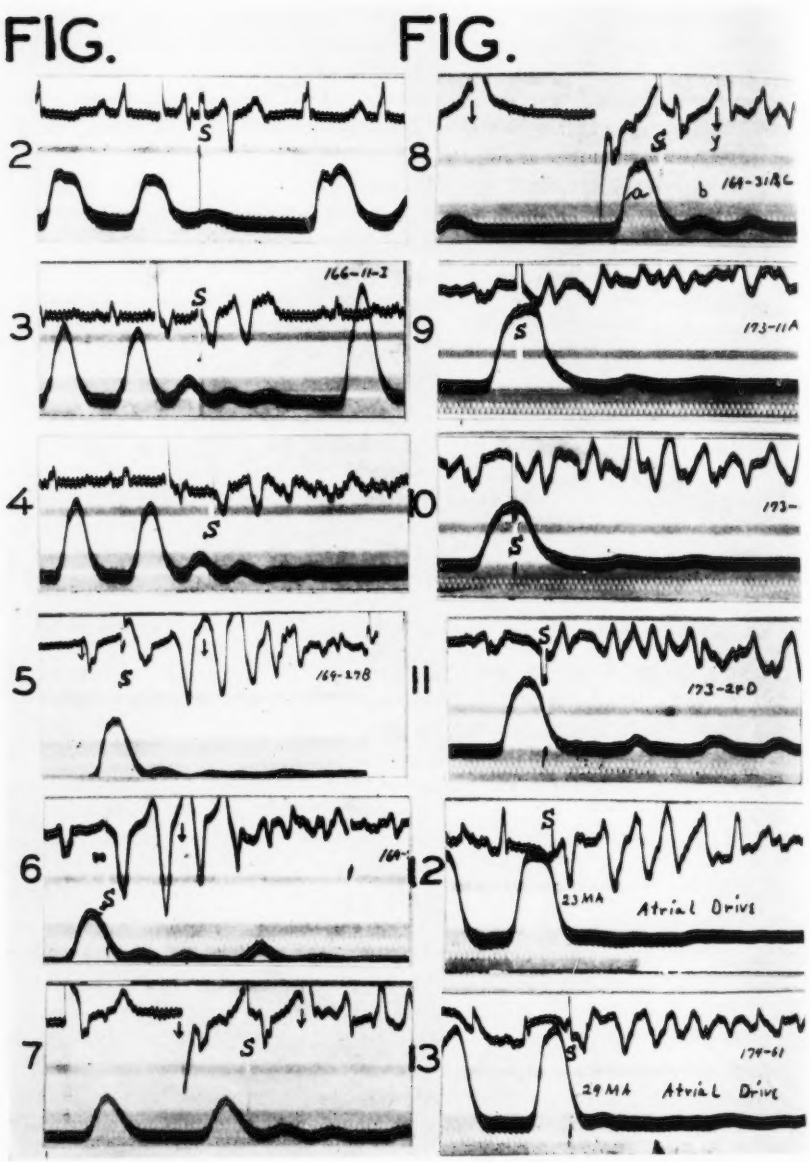
As in studies previously reported (1, 2, 3, 4) dogs were anesthetized with morphine and barbital, the heart was exposed and Ag-AgCl electrodes 8 mm. apart were applied to an easily identifiable spot. After each fibrillation, dogs were resuscitated by use of the Hooker countershock method. A "standard lead" electrocardiogram, a left ventricular pressure curve and the incidence, duration and strength of the D.C. shock were simultaneously recorded.

Results. Our interpretations are based on a thorough study of many sets of observations on 15 dogs.

Span of reactive and vulnerable period in small ineffective beats of left ventricular origin. Small ineffective beats are characterized by pressure curves having a slowly rising gradient, a rather peaked summit, and a gradually declining gradient. Since no expulsion of blood from the left ventricle occurs, the end of mechanical systole cannot be definitely determined.

In such beats the refractory state terminates definitely before the peak, i.e., a second stimulus applied somewhat before the top, on the ascending curve, causes another small contraction (fig. 2). However, multiple beats or fibrillation only occur when the shock falls on the descending limb. In other words, the *vulnerable period* lies beyond the summit and extends nearly to the bottom of the descending limb. Curves illustrating such effects are shown in figures 3 and 4. In this experiment 15 D.C. shocks (0.0066–0.02 sec. in duration, 9.5–10 M.A.), administered 0.026 second before the peak caused 1 additional beat; 16 D.C. shocks (0.01–0.02 sec. in duration, 9–10 M.A.), applied just after the peak, caused a single beat, 10 times; two beats, 4 times; and no response, twice. Six shocks (0.0068–0.018 sec. duration; 9–10 M.A.) caused two premature contractions, 4

[3] The resistance K, figure 1, of the communication (2) was erroneously labeled. It should have been 35,000 ohms.



Figs. 2–13. Segments of records showing in order e.c.g. (lead I), stimulation shock and left ventricular pressure curves. Time, 0.02 second. Discussion in text.

times; a single premature beat, once; and finally fibrillation, as shown in figure 4.

Similar reactions occurred when the left ventricle was driven rapidly and a brief D.C. shock was occasionally introduced at various moments of a cycle. A few of the fibrillations following use of strong shocks of 30 M.A. are illustrated by curves of figures 5, 6 and 7. While such current intensities represented the fibrillation thresholds early in an experiment, it was found after repeated fibrillations and defibrillations in which adequate recovery intervals were not permitted, that much weaker currents sufficed. An extreme instance is shown in figure 8 from the same experiment in which a shock of only 6 M.A. eventually caused fibrillation. The question may be raised whether the D.C. shock *S* or the succeeding condenser shock which fell at *Y* actually caused the fibrillation. While it seems improbable that such weak condenser shocks could have been effective, it is really immaterial, for, in either case, fibrillation was caused by a weak shock during a vulnerable period.

Span of reactive and vulnerable periods in large, effective beats of ventricular origin. We found in large beats of left ventricular origin that a suprathreshold shock (ca. 20-30 M.A.) induces fibrillation when it is applied anywhere between midsystole to the end of isometric relaxation. This is in contrast to supraventricular beats in fresh hearts which cannot be fibrillated by shocks applied far down on the descending limb. This apparently indicates an extension of the vulnerable period. Typical fibrillation produced by shocks (0.02 sec., 22-23 M.A.) applied during various moments of the cardiac cycle are shown in figures 9, 10 and 11.

Such extension of the vulnerable period almost to the bottom of the isometric relaxation curve is not wholly a function of an ipsiventricular focus of excitation. In previous work we had noted occasionally after repeated fibrillations and defibrillations that shocks administered somewhat later than the limits originally placed on the span of the vulnerable period (1) caused fibrillation. We have now obtained more striking confirmation of the fact that repeated fibrillations and revival, or deterioration of the myocardium, cause a similar extension of the vulnerable period in normally initiated beats. This is illustrated by two records taken respectively early and late in a prolonged experiment. In the record of figure 12 the latest moment when fibrillation was elicited (D.C. shock 0.02 sec., 23 M.A.) was near the end of systole. In the record of figure 13 a similar shock applied low on the descending limb of the curve was quite effective. Comparison of the ventricular pressure curves reveals fully as large an amplitude in the latter; but the duration of systole is obviously less. The latter is an early characteristic of many deleterious actions such as anoxia, fatigue, etc.

Comparative fibrillation thresholds of left ventricular beats elicited over

normal and aberrant pathways. The fibrillation threshold was measured, as in previous work (4, 6), by the recorded strength of a D.C. shock (0.02 sec. in duration), which was just sufficient to cause fibrillation. Such comparisons are only valid when the normally and aberrantly excited beats have similar durations. This was achieved by clamping the sinus node and driving the heart at a definite tempo, first by electrodes applied to the right and then to the left ventricle. It may be added, parenthetically, that previous studies (7) had shown that such right ventricular stimulation causes the left ventricle to be excited *via* the left bundle branch, as in supraventricular rhythms. Aside from convenience, it has the advantage over an atrial drive of the heart, that A-V conduction disturbances are avoided at rapid rates.

In addition to numerous preliminary tests, crucial experiments were carried out on four dogs, in which adequate time for equilibration was allowed (cf. ref. 6). The results obtained gave no evidence of any significant difference in the fibrillating threshold of left ventricular beats induced by left or right ventricular stimulation.

Discussion. Our results indicate 1, that even in fresh hearts the vulnerable period of premature beats is extended nearly to the end of the isometric relaxation process, but 2, that the fibrillation threshold is not significantly altered. The first demonstration supplies supplementary evidence in harmony with our conception of the induction of fibrillation following coronary occlusion (4). On the other hand, we cannot confirm our interpretation that weak, prolonged, direct currents induce fibrillation when opening of the current occurs during the vulnerable period of a premature systole which has a reduced threshold.

Our results are also interesting in crystallizing our conception as to the ultimate processes which underlie the initiation of fibrillation. An asynchronous offset of fractionate contractions, caused either by a slight delay in their onset or by variations in their durations is, as King (8) has emphasized, considered essential to any concept as to how fibrillation starts. Our results certainly fail to show that a greater degree of asynchronicity in termination of contractions in premature beats reduces the fibrillation threshold. The sensitivity to fibrillation therefore seems to depend rather on some inherent characteristic of cardiac muscle at the beginning of the relaxation of its ultimate units. This is supported by our observations that the period of vulnerability is extended in beats which arise from an ipsiventricular focus. That this is not solely due to greater differences in the termination of fractionate contractions—as we had postulated—is indicated by the facts 1, that the degree of extension is too great, and 2, that a similar extension occurs in normally excited ventricles with impaired function.

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ACTIVITIES OF SINGLE MOTOR UNITS IN MAN DURING SLIGHT VOLUNTARY EFFORTS

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Despite the vast literature concerning voluntary contractions of human muscles, there has not yet been presented an adequate description of the discharges from individual nerve cells of the spinal motor horn as they participate in the production of the various movements which occur. In this paper we are presenting material which deals with the responses of those fibers in a muscle which are innervated by one or by a very few motor nerve cells. The spinal motor neurone, which is the final common path (Sherrington, 1) for the activation of skeletal muscle has for its functional element the motor unit. This was defined by Liddell and Sherrington (2) as the " . . . motoneurone axone and its adjunct muscle fibres. . . . " Adrian and Bronk (3) and Denny-Brown (4) showed that it was possible to record the electrical discharges from single motor units which were responding during reflex activity and Adrian and Bronk recorded discharges from single units of muscles participating in voluntary contractions. Later Smith (5) and Lindsley (6) recorded the responses of single motor units in voluntary activity. Their work established a foundation on which we have attempted to place the beginning of a more complete structure.

In selecting the experimental material which is to be considered below the attempt has been made to use records which would yield an outline description of the activities of single motor units under conditions ranging between threshold and slight to moderate voluntary effort. For the moment we shall avoid detailed quantitative considerations and shall describe the single unit response first as it occurs in quick movements of various intensities, either as isolated single efforts or as a rhythmic series of movements; second, as it participates in sustained movements begun or ended more or less suddenly; and third, as its activities are related to the activities of other near-by motor units whose responses may also be recorded.

METHODS. Action potentials have been recorded on bromide

paper by means of an oscillograph galvanometer of the Duddell type. The galvanometer is activated by a differential amplifier. For lead electrodes we employ three fine steel sewing needles (no. 12). These are lacquered to the extreme tips, sterilized in phenol solution, rinsed in alcohol and inserted into a muscle through a prepared skin area. In most cases the needles are arranged in a triangle about 2 mm. on a side. These electrodes are not satisfactory if intense muscular contractions are produced. They are, however, well suited for use with the slight tension efforts which we have studied. Following each experiment the needles are sharpened and relacquered. They cause but slight discomfort as they are being inserted into the skin and once placed in the muscle should not be noticeable to the subject. Occasionally a needle impinges on a nerve twig and causes pain or muscular twitches with each movement. In these cases the needle is removed and reinserted. A telephone receiver connected with the amplifier furnishes information as to the proximity and activity of muscle units near the needle point and thus acts first as a guide in placing needles and thereafter as an indication to the subject concerning the responses which are being recorded during the tension efforts which he may make. Occasionally we have obtained excellent records of single unit responses with quite uninsulated needles, the ground lead and one grid needle being thrust into the skin and the other grid needle being inserted just deeply enough to record from a unit which lies near the surface of the muscle. This method is not recommended as routinely dependable.

Although single unit responses can sometimes be gotten by other types of electrode, it seems pertinent to point out here that neither the use of the coaxial type of electrode nor the use of "pore" type electrodes such as we have used, guarantees recording from discrete single units. Each record must be interpreted without preconceptions or prejudices as to the specificity of lead relationships for a given type of electrode. In our experiments we have usually placed the needles so that with slight movements only the responses of a single unit are apparent in the records. In almost all cases the responses of additional units appear when somewhat more intense efforts are made. Under favorable circumstances we can obtain records with one, two or three units showing responses which are reproducible and well controlled. Such records have been used in examining the relationship between activities of separate units. With larger numbers of responding units recording we have found it impractical to follow the activities of a given single unit. It is, of course, largely a matter of chance to place the needle points so that a single unit may record without the complicating presence of other near-by units which are

also active. Although, as will be seen below, the different units of a muscle probably keep rather constant threshold relationships for a given movement pattern, the various units of that muscle do not all begin activity at the same effort threshold. Consequently a single unit whose activity is being recorded may be one which comes into activity with a minimum tension of the muscle concerned or it may come into activity only after the muscle has developed considerable tension. Moreover this threshold may be changed by such relatively slight differences in the neuromuscular pattern as may be brought about by changes of limb position or of general muscular tension. In many instances a movement has been found with which the recording unit is brought into activity with a minimum of volitional effort, the subject performing a quick and most delicate tensing of the muscle. In such records, even at high amplifications, the background noise has been so low as to indicate that few if any other near-by units were participating in the effort.

In no muscle have we found a predictable distribution of muscle units of lower or of higher response thresholds. We have frequently obtained excellent records with an active needle near the surface of a muscle or near its tendonous end but we have also obtained excellent records from the muscle belly. However, the main requirement for a readable record is that a unit close to a recording needle shall become active at a tension level considerably below that at which closely adjacent units become active. With a fairly random distribution of thresholds for different units throughout the muscle such a requirement as the foregoing might be met most readily at the muscle surface. It seems probable therefore that the apparently optimal situation for recording sometimes found at a muscle surface is a matter of statistical rather than of functional anatomy. Likewise we believe it to be largely a matter of sampling that we usually find, as did Smith, that the first units to be heard responding as the needle is thrust into the muscle are relatively remote from the lead needle and consequently give but a faint click in the telephone or a low amplitude of galvanometer excursion. It is entirely possible that there may be a functional organization of units within a muscle. However, the nature of such organization has not yet been demonstrated.

As regards the constancy of spike heights recorded from single unit responses, the accompanying records are typical of those which we have obtained. Frequently records have shown but a few per cent difference of spike heights with wide ranges of the tension effort and of the resulting unit discharge frequency. The record of figure 1-K shows a considerable fluctuation of recorded spike heights. Because we have found, as have others previously, that slight changes in needle position may result in

marked changes of recorded amplitude, we have attributed such changes of amplitude as are seen in figure 1-K to mechanical disturbance of the spatial relationships between the active unit and the lead needles. We have found no reason to believe that there is an increase of the amplitude of the single unit spike with increased tension or frequency of unit discharge. An increase of "spike" amplitude with increased tension is, of course, seen where multiple units are recorded so that with increased tension and increased number of recording units there is increased summation of unit discharges.

Records of tension exerted have been obtained by use of spring torsion levers. Because the recorded movement of a part has usually involved the participation of more than one muscle, the records so far obtained have been of service merely as guides to time and tension relationships. Records for finger muscle contractions have been the most satisfactory in this respect, but even these cannot be regarded as precision records of the tension changes of a single muscle.

RESULTS. 1. *Responses with brief efforts.* It has been a generally held belief that even the shortest of volitional efforts involves a brief but rhythmic discharge of those motor neurones concerned in the activation of muscle fibers. In recent years it has seemed clear that in sustained voluntary contractions the individual motor neurones discharge more or less asynchronously but at relatively slow rates. For a sustained movement, the discharge frequency for a given motor neurone may for the moment be considered to range upward from Lindsley's minimum figure of 3 per second. Stetson and Bouman (7) used skin electrodes to record action potentials from the muscles in the forearm while tapping movements were being made with the hand. They reported a tendency for action potentials to be grouped into unit bursts which had a duration of about 50 msec. Since sustained discharge of single motor units at rates less than 20 per second (that is, with discharge intervals greater than 50 msec.) are easily obtained, there seemed to be ample possibility of making a volitional movement so brief and so slight that a single recording motor unit might respond once and only once for each volitional effort.

This has been attempted and found possible. Eight normal individuals have so far acted as subjects. Each of these individuals has yielded records from one or more of fourteen different muscles. In all cases it has been possible to record single motor unit discharges with discrete volitional efforts. Figure 1-A illustrates the case in point. While this record was being made the finger could exert a steady tension of about twenty grams on the lever without discharge of the recording unit. For the first and last two responses of figure 1-A the finger made a quick flexion movement representing a further tension on the lever of about ten grams. The movement at the finger was about $\frac{1}{7}$ mm. Comparison of the elec-

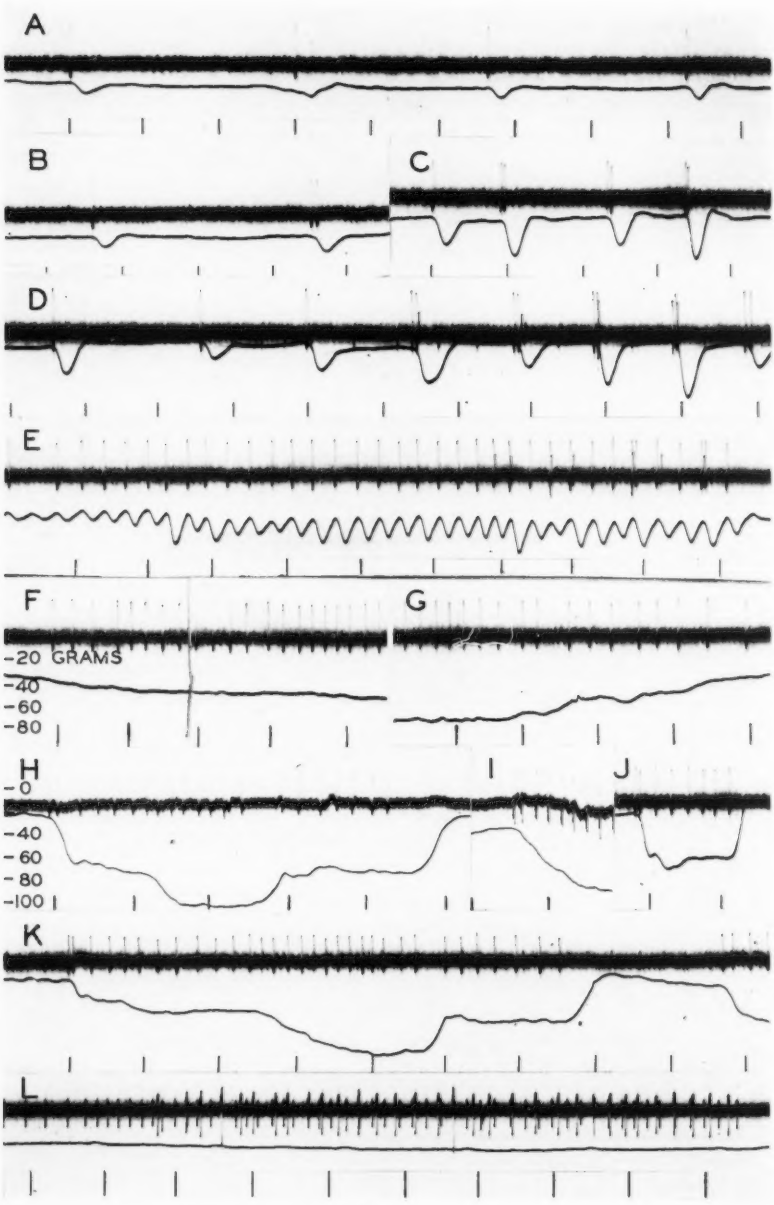

Fig. 1

trical and mechanical records shows that each tension effort produced a single spike discharge which is interpreted as representing the discharge from a single motor unit. Such an interpretation is based on the fact that if the tension is not quickly released but is sustained, there occurs the simple rhythmic discharge of recorded spikes of essentially constant height and form. This criterion of single unit activity has been considered acceptable by previous workers. It does include certain assumptions which are made in this as in previous studies by other workers. Single spike (i.e., single unit) discharges may also be obtained when the subject makes a slow tensing effort until the telephone indicates a discharge, the effort then being immediately discontinued (fig. 1-A, second response). Because the subject's reaction time is involved in such a procedure he must be in a good state of motor control if his attempt is to be successful. On the other hand, under favorable conditions, this is perhaps the most dependable method of eliciting such single spike responses. In a subject under general tension the method usually produces only multiple spike discharges.

To complete the record it may be said that single spike responses have been produced in two other ways. The first of these has consisted in the

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Fig. 1. All records except H, I and J were taken from the flexor digitorum sublimis over a period of about two hours. H, I and J were recorded from an adjacent region of the same muscle at another time. Time lines, inked for reproduction, mark intervals of $\frac{1}{2}$ second. Mechanical record from spring lever attached to finger by a thread. Mechanical movement amplified about 20x. Spring lever tension for records H, I and J indicated on record H. Tension calibration for other records indicated on record F.

A. Discrete volitional movements resulted in single unit discharges for each movement.

B and C. With greater effort the single unit shows a double discharge.

D. With still greater effort additional units near the lead-off point become active.

E. Response of single unit with repetitive movement. Toward the latter part of the strip a second unit is responding.

F and G. Slow increase and decrease of unit discharge frequency with slow increase and decrease of tension, respectively. In G, the last discharge of the unit is included in the figure. There followed a period of several seconds during which the recording unit showed no discharges.

H. Double discharge followed by a pause at the beginning of a movement. Interruption of discharge with quick partial release of tension and sudden cessation of discharge with quick final tension release.

I. Double discharge with duration of second interval nearly equal to that of the third when initial tension increase is smoothly continued.

J. Double discharge without long pause with but brief interruption of tension increase.

K. Record of flexion of third digit.

L. Record of flexion of fourth digit immediately after recording of K. Position of needles unchanged from that for K.

development of a brief instant of muscular resistance when a quick "passive movement" is applied by the subject himself. A highly coördinated resistance effort is thus permitted. A second method has occasionally been used when there has existed a tonic "resting" activity of the unit recording. This "resting" discharge has been due to the effort of maintaining immobility of a limb which has not been properly supported. Under these conditions the "resting" discharge of the recording unit can sometimes be stopped by an appropriate antagonistic effort. During a momentary release of such an antagonistic effort a single discharge of the recording unit may occur.

If the subject makes a quick effort of somewhat greater intensity than that considered above, double discharges may appear. Figures 1-B and 1-C illustrate such responses. The records are typical of many which have been obtained. Although the spike heights are frequently not the same it seems probable that they represent discharges from the same unit. The reasons for this interpretation follow. First, an equal or greater dissimilarity of spike height may be seen during repetitive discharges of the same unit during sustained effort. Second, in many cases the unit of next higher threshold to appear in records of greater tension efforts shows grossly different spike height or form. Third, because the double responses seen in our records are similar in occurrence to the double responses recorded by other workers, as for example, by Denny-Brown for crossed extension reflexes and interpreted by him on a basis of still further evidence as responses of the same unit.

With quick efforts of somewhat greater intensity (fig. 1-D) the two spikes of the double response fall more closely together and the responses of other units also begin to appear. The units of lower threshold appear to show increasingly shorter latencies so that the record may show a marked summation of spike potentials. Occasionally the spikes of the double response have been separated by as little as 10 msec., a value in accord with that found by Denny-Brown in certain of the reflex responses in his experiments. If a rhythmic series of discrete efforts are made, a single unit response may be recorded for each movement (fig. 1-E). With an effort to achieve too high a rate of alternation, the movement becomes less well controlled and additional units are introduced into activity, with or without the appearance of the double spike responses. It is to be seen from the above that a sudden movement may be expected to have a high degree of muscular effectiveness because of the approach to simultaneity of discharge of the more promptly responding muscle fibers and because the double discharge of some of the responding units will fall within time limits which will yield considerable mechanical summation. On a basis of our experiences, it would seem probable that the quicker of the tapping movements employed by Stetson and Bouman might well have produced

a single discharge of the units of higher threshold and a double discharge of units of lower threshold.

2. *Responses with sustained efforts.* Several features typical of the single unit response during the beginning, maintenance and ending of a sustained tension are indicated by figure 1-F to 1-J, inclusive. If the tension is developed smoothly there is a gradual increase of discharge frequency to the maximal rate attained and then a gradual decrease in frequency as the tension is slowly released. Under such conditions there may be rather close agreement between the tension levels at which the unit discharge begins and that at which it ends. Such a case is seen in figures 1-F and 1-G. These figures were recorded at the beginning and ending of a sustained movement, the intermediate part of the record covering several seconds of the effort being omitted. A frequent type of start is that indicated by figure 1-H in which there is seen an initial double discharge, a long pause and then continued discharge. The long pause, *in our material*, has been associated with a brief interruption of the tensing effort. This does not seem to apply, for example, to certain of the published reflex responses recorded by Eccles and Hoff (8). If, in our experiments, the tension interruption does not occur the long pause may be absent.

Cessation of discharge may be gradual as in figure 1-G or abrupt as in figures 1-H, 1-J and 1-K. If there is a quick release of tension from a higher to a lower tension level (figs. 1-H and 1-K) there is a brief cessation of discharge followed by a new discharge rhythm at a lower frequency. It is apparent that with sudden onset and cessation of activity there is no necessary quantitative relationship between the lever tensions at which the unit discharge begins or ends.

For a tension achieved quickly and then maintained constant, the early responses are followed by a continuous discharge which, at slight tensions, may continue almost indefinitely. If the responses of several units are being recorded it is seen each unit will discharge at its own rate so that the responses of the various units quickly become completely asynchronous. In such a sustained tension a given motor unit will continue its discharge without interruption or alternation as long as the tension is sustained and provided that the motor pattern is unchanged. It is difficult to continue a smooth tension effort without change of the movement pattern and such maintained efforts have not been made for periods longer than three minutes. On several occasions tension efforts sustained for one to two minutes have been made repeatedly over a period of thirty to forty minutes. The records were comparable in all cases. In one case such sampling was repeated over a two hour period.

3. *Relationships between activities of different units.* Threshold and discharge relationships between different units in the same muscle have

been discussed by various authors. The possibility of rotational activity of motor units suggested by Forbes (9) dealt with a situation involving a frequency of motor fiber discharge much greater than that which has been indicated by the later work on single motor units. The work of Denny-Brown, of Smith and of Lindsley has shown that a single motor unit set into activity, whether by reflex activity or by voluntary motor effort, will continue to show a rather constant rate of discharge without interruption as long as the smooth maintenance of tension continues. Within the limits of interpretation permitted by the sampling methods which these authors have used, their work may well be considered to indicate that alternation of motor units, rotational activity, or haphazard and irregular changes in unit activity do not occur during a sustained or smoothly changing effort. Recently Hoefer and Putnam (10) have presented the conclusion that (p. 218) " . . . individual motor units are independent in their frequencies of each other . . . " so that " . . . the individual units may alternate in their activity. . . . " The records from which their discharge frequencies and "unit" relationships were determined appear to have been in considerable part typical records of responses of multiple units (as, for example, their fig. 3, lower record of each strip). Consequently the high unit frequencies and the apparent independence of the activities of the different units which they report may well be held subject to question.

We have undertaken to obtain data dealing with this point. Our first method of procedure yielded results in complete confirmation of Lindsley's observation that a single unit may be kept active for long periods of time with no sign of its dropping out, as would be expected if there occurred any rotational or alternating activity. Moreover with repeated trials, starting, maintaining, stopping and again starting the tension effort, the same unit repeatedly became active before other units and continued in action throughout the period of tension even though other units might have been brought into activity when the tension was increased to levels above that which was threshold only for the first unit. An example of this latter situation is seen in figure 1-L where a unit which may be designated as A appeared with a lever tension lower than that at which a second unit, B, became active. However, unit A continues discharging regularly even after unit B becomes active. A further complication was first noticed in a record being made from the biceps muscle of the arm where it was first thought that at times one and at times another motor unit responded at lower tension efforts. More careful attention to the movement showed that both units were responding at very close to threshold for forearm flexion. However, the one unit showed a slightly lower response threshold when the movement was flexion with pronation and the other unit showed the lower response threshold when the movement was

flexion with supernation. Similar results were obtained on another occasion with the lateral division of the triceps muscle of the arm.

Figures 1-K and 1-L illustrate another instance of the same sort. Needles were inserted in the superficial flexor muscle to the fingers. The needle points were separated by about 4 mm., one being somewhat deeper than the others. Movement of the index or little fingers gave no recorded activity, even with rather intense flexion effort. Flexion of the third finger produced threshold recorded activity as indicated in figure 1-K and flexion of the fourth finger gave threshold recorded activity as recorded in figure 1-L. To obtain the mechanical records which are reproduced, a thread loop attached to the recording lever was carefully changed from one finger to the other by an assistant. However, the procedure of recording cannot be held responsible for the shift of the unit recording because of shift of the needle positions as the transfer was accomplished with little or no disturbance and was repeated several times. Moreover, it was observed that a similar change of threshold unit response occurred when the fingers were alternately flexed either free or against an unyielding surface and with no attachment to the spring lever. For figures 1-K and 1-L it is obvious that the recording units were significantly closer in the one case to one of the needles and closer to the other needle in the other case. In two of our records it seems probable that a similar functionally different pair of units lay mainly within the very limited range of lead of a single needle point but such an interpretation must for the moment be held as merely tentative. For the case of figure 1-K and 1-L it is recognized that the superficial finger flexor muscle is not a simple muscle and that movement of the third or of the fourth fingers may be made more or less independently. For this case, therefore, we could merely conclude that for the position of the needles which happened to apply, the recorded response of "flexing the fingers" might well have indicated an apparent independence of threshold of the units involved. On the other hand, carefully repeated movements of a single digital joint showed consistent and reproducible responses.

For the present it may be said without qualification that in none of our records, obtained when both subject and operator have been satisfied that the movement pattern has remained unchanged has a unit, A, appearing at a tension threshold lower than that of a second unit, B, shown any failure to continue in activity under maintained tension effort. Usually A will at all times have a frequency of discharge higher than that for B. This is not invariable in our records, however, though the frequency of B has never been more than slightly higher than that of A and in these cases the effort has always been sufficiently great so that there has always been the possibility of an unconscious slight change in the movement pattern. Lindsley's summary (p. 98) of the means by which the strength of a muscle

contraction may be graded may therefore be used with but slight modification to describe the means by which a muscle may slowly and smoothly develop a contraction of considerable tension. The movement begins with a slow response of a very few units perhaps even of a single unit. As the contraction increases these units increase in the frequency of their discharge and other units are brought into action. When the tension increase is halted and the effort is continued as a smoothly sustained tension, all the responding units continue in their asychronous, rhythmic discharge probably until voluntary effort of a postural or other change disturbs the total movement pattern.

SUMMARY

1. Electrical records have been obtained from one or a few units of normal human muscle under various conditions of slight voluntary effort.

2. Discrete, slight and brief voluntary efforts may each be accompanied by a single discharge of the motor unit whose activity is being recorded.

3. For quick movements of slightly greater force there may be double discharges of a single recording unit.

4. Rhythmic movements, repeated several times per second, may each show a single response of a single recording unit.

5. With more intense, quick movements additional motor units are brought into activity. The more intense the movement the more are summated potential spikes to be observed in the initial phase of the response.

6. Sustained movements may be begun slowly with a gradual increase of discharge frequency of the units of lowest threshold and with a gradual accession of additional units. If the movement is begun suddenly there is usually a double discharge of the units of lowest threshold and an approach toward a simultaneity of discharge of the various units. Following this first burst the discharge of the various units quickly becomes quite asynchronous.

7. Cessation of movement may be due either to gradual or to sudden cessation of unit activity.

8. No records have shown rotation or alternation of unit activity in sustained tension efforts provided that the movement pattern has remained unchanged.

9. No evidence has been observed which would seem to support the statement that there is increased amplitude of the single unit spike with increased muscular tension.

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THE INFLUENCE OF COLD AND HEAT ON THE VAGO-INSULIN AND THE SYMPATHETICO-ADRENAL SYSTEMS

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Since Cannon's investigations the effect of cold on the sympathetico-adrenal system has been well established. The importance of this reaction is evident from the fact that sympathectomized cats are more sensitive to cold than unoperated controls (Sawyer and Schlossberg). Hyperglycemia which regularly follows the exposure to cold is abolished by splanchnicotomy (Geiger). The effect of increased environmental temperature on the autonomic nervous system is less known. Although Geiger observed that heating the blood in the carotid arteries leads to a discharge over the vago-insulin system which was abolished by vagotomy he was unable to find consistent effects of increased environmental temperature on the blood sugar. In most of his observations hyperglycemia occurred which was thought to be due to emotional excitement. In view of the fact that procedures and drugs such as anoxia, conditions leading to emotional excitement, metrazol, cocaine and electrically induced convulsions (Feldman, Cortell and Gellhorn; Kessler and Gellhorn) cause an excitation of both vago-insulin and sympathetico-adrenal systems, it was deemed of interest to investigate the relation of heat and cold to these systems.

METHOD. The methods used were similar to those employed in preceding reports. The experiments were performed on four groups of rats: first, normal; second, adreno-demedullated rats; third, adreno-demedullated-vagotomized rats; fourth, vagotomized rats. Prior to the experiment the animals were fasted for 16 hours. Cold was applied by immersing the animals for 10 minutes in water of 2 to 4°C. The environmental temperature was raised to 31 to 32°C. and maintained at this level by means of electric light bulbs. This mild degree of heating did not cause any visible signs of increased excitability in these animals. They appeared rather lethargic. The blood sugar was determined by means of the Somogyi modification of the Shaffer-Hartman method.

RESULTS. Table 1 shows not only the familiar rise in blood sugar

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following cooling in the normal rat which is generally attributed to the effect on the sympathetico-adrenal system but indicates also that the

TABLE 1

Effect of cold on blood sugar*

Blood sugar (mgm. per cent)

RAT NUMBER	CONTROL (BEFORE COOLING)	TIME AFTER COOLING		
		1 minute	60 minutes	120 minutes
A. Normal rats				
1	74	90	101	99
2	71	80	91	91
3	71	84	99	87
4	74	81	90	85
5	73	86	101	91
6	72	89	110	100
Mean	73	85	97	92
St. dev.	1.3	3.9	5.2	5.6
P		<0.01	<0.01	<0.01
B. Adreno-demedullated rats				
1	66	49	43	52
2	67	60	49	53
3	62	56	54	48
4	60	60	52	38
5	59	48	41	42
6	62	60	49	43
Mean	63	56	48	46
St. dev.	2.7	4.9	4.5	5.4
P		0.011	<0.01	<0.01
C. Adreno-demedullated-vagotomized rats				
1	71	79	75	75
2	67	70	77	72
3	71	81	80	77
4	65	67	66	63
5	66	71	75	69
6	69	71	73	71
Mean	68	73	74	71
St. dev.	2.5	5.1	4.4	4.5
P		0.05	0.02	0.15

* The rats were placed in an ice water bath of 2 to 4°C. for 10 minutes.

vago-insulin system is excited. This is proven by the fact that in the adreno-demedullated animals exposure to cold leads to a consistent fall

TABLE 2

The effect of heat (31–32°C.) on the blood sugar

RAT NUMBER	CONTROL		1 HOUR		2 HOURS		4 HOURS		5 HOURS		6 HOURS	
	Blood sugar	Body temperature	Blood sugar	Body temperature	Blood sugar	Body temperature	Blood sugar	Body temperature	Blood sugar	Body temperature	Blood sugar	Body temperature
	mgm. per cent	°F.	*mgm. per cent*	°F.	*mgm. per cent*	°F.	*mgm. per cent*	°F.	*mgm. per cent*	°F.	*mgm. per cent*	°F.
A. Normal rats												
1	73	100	79	104	67	104	59	104	61	104	63	104
2	74	100	75	103	80	103	70	103	60	104	63	104
3	71	102	75	104	76	104	72	104	65	104	61	104
4	75	103	79	104	81	104	71	104	66	104	60	104
5	74	102	77	102	77	102	68	103	69	104	69	103
6	71	101	75	102	81	103	71	104	67	104	63	103
Mean	73		77		77		68		65		63	
St. dev.	1.6		1.5		4.9		4.4		3.0		2.7	
P			<0.01		0.1		<0.01		<0.01		<0.01	
B. Adreno-demedullated rats												
1	63	99	63	100	58	102	56	104	54	104	58	104
2	69	100	60	102	53	103	56	103	58	104	61	104
3	69	101	60	102	56	103	48	104	54	104	58	104
4	65	99	67	100	59	101	56	102	59	103	62	104
5	68	98	71	100	65	101	59	103	60	103	65	104
6	66	99	58	101	56	102	45	103	47	103	55	103
Mean	67		63		58		53		55		60	
St. dev.	2.1		4.4		3.7		4.9		4.4		3.2	
P			0.13		<0.01		<0.01		<0.01		<0.01	
C. Adreno-demedullated-vagotomized rats												
1	67	100	77	102	97	102	88	103	86	103	86	103
2	63	100	67	102	71	104	67	104	67	103	67	103
3	74	102	77	102	77	102	68	103	69	104	69	103
4	66	98	67	102	71	102	69	104				
5	65	99	88	103	73	104	76	104				
6	75	100	80	102	85	102	79	102	79	103	77	103
Mean	68		76		79		74		75		75	
St. dev.	5.3		7.5		9.3		7.9		7.8		7.7	
P			0.07		0.04		0.15		0.16		0.13	

TABLE 2—*Concluded*

RAT NUMBER	CONTROL		1 HOUR		2 HOURS		4 HOURS		5 HOURS		6 HOURS	
	Blood sugar	Body temperature	Blood sugar	Body temperature	Blood sugar	Body temperature	Blood sugar	Body temperature	Blood sugar	Body temperature	Blood sugar	Body temperature
D. Vagotomized rats												
	mgm. per cent	°F.	*mgm. per cent*	°F.	*mgm. per cent*	°F.	*mgm. per cent*	°F.	*mgm. per cent*	°F.	*mgm. per cent*	°F.
1	70	100	74	103	86	103	87	104	81	104	72	104
2	73	100	79	103	84	103	89	104	86	104	76	104
3	71	100	80	102	90	103	90	103	83	103	73	104
4	72	99	76	101	88	102	87	103	81	103	77	104
5	71	98	75	104	81	104	72	104	72	104	71	104
6	76	99	81	101	88	102	85	102	83	103	81	103
Mean	72		77		86		85		81		75	
St. dev.	1.7		2.3		3.2		6.1		4.3		2.4	
P			<0.01		<0.01		<0.01		<0.01		0.11	

in blood sugar. This effect is masked by the predominance of the sympathetico-adrenal effects in the normal animal. That this interpretation is correct is proven by the fact that no significant changes in blood sugar occur if neither the adrenal medulla nor the islands of Langerhans can be excited via the autonomic nervous system. This is shown in group 3 in which the adrenals have been demedullated and the vagi cut and in which only a very slight and statistically insignificant rise in blood sugar occurred.

Table 2 shows the effect of increased environmental temperature on the blood sugar of rats. Our procedure raised the temperature in rats by about 4°F. during a period of 6 hours. Although no significant difference in the reaction of the temperature of the body was observed in these various groups, the blood sugar response was specific. In order to understand the relatively complex reaction of the normal rats it is best to consider first the effect of heat on group B (adrenodemedullated), and D (vagotomized). In the former the blood sugar can be influenced as far as nervous impulses are concerned only by stimulation of the vago-insulin system and in the latter solely by stimulation of the sympathetico-adrenal system. The results correspond to this expectation in as much as the blood sugar falls on exposure to heat in adreno-demedullated rats, whereas heat caused a hyperglycemia in vagotomized animals. These results make it probable that no significant changes in blood sugar would result from heat if the nervous action on both insulin and adrenal systems were no longer possible. Experiments with group C (adreno-demedullated-vagotomized) show

indeed only a slight rise in blood sugar which is statistically insignificant. Moreover, rats kept at room temperature and subjected to the same number of blood samplings do not show any significant change in blood sugar (table 3).

The blood sugar curve of normal rats shows on exposure to heat a significant and progressive fall beginning with the fourth hour. During the first 2 hours there is a slight and statistically not significant rise in blood sugar. In the light of the experiments performed on vagotomized and on adreno-demedullated rats it seems probable that the curve obtained on normal rats results from the interaction of the excitation of both sympathetico-adrenal and vago-insulin systems. Apparently increased as well as decreased environmental temperature leads to discharges over the sympathetico-adrenal and vago-insulin systems, but there is a fundamental difference in the reactivity of the normal animal to cold and to heat.

TABLE 3

Normal rats kept at room temperature (23°C.)

Blood sugar (mgm. per cent) determined in hourly intervals

RAT NUMBER	0 HOUR	1 HOUR	2 HOURS	3½ HOURS	4 HOURS	5 HOURS	6 HOURS
1	74	76	75	77	77	75	76
2	77	77	75	78	76	77	75
3	75	73	74	77	75	76	74
4	72	75	74	76	74	75	75
Mean	75	76	75	77	76	76	75

Whereas on exposure to cold the effect on the sympathetico-adrenal system predominates to such an extent that excitation of the vago-insulin system occurring simultaneously with that of the sympathetico-adrenal system can only be revealed by the study of the adreno-demedullated rats, heat acts more powerfully on the vago-insulin system although the excitation of the sympathetico-adrenal system occurring in normal rats at the same time leads to a delay of the fall in blood sugar in these animals. If, however, effects on the vago-insulin system are excluded by the subdiaphragmatic vagotomy, the action of heat on the sympathetico-adrenal system is easily ascertained by its hyperglycemic effect.

Cold, heat, anoxia, metrazol, emotional excitement, bulbo-capnine, cocaine and electrically induced convulsions act on both vago-insulin and sympathetico-adrenal systems. In contrast to all the other procedures just mentioned heat is the only condition thus far investigated in which the vago-insulin system is more excited than the sympathetico-adrenal system if the change in blood sugar is taken as an indicator. This specificity is

important with respect to homeostatic regulations. Dudley and collaborators showed a decrease in oxygen consumption after insulin. It is also known that, particularly in man, insulin hypoglycemia is associated with a fall in body temperature. On both effects, however, the literature is controversial, probably due to the fact that during insulin hypoglycemia adrenalin is being secreted and may offset the effect of insulin on metabolism and body temperature. There is further evidence in the studies of Laufberger, Rosenthal, Licht and Freund that insulin interferes with heat production. In the light of these observations the predominance of the stimulation of the vago-insulin system on exposure to heat seems to have a tendency to counteract the deleterious effects of over-heating just as the predominant action on the sympathetico-adrenal system counteracts the harmful effects of cold not only by vaso-constriction but also by increasing heat production.

SUMMARY

Normal (A), adreno-demedullated (B), vagotomized (C), and adreno-demedullated-vagotomized (D) rats were exposed to cold by immersion in water of 2 to 4°C. for 10 minutes or to heat by keeping them at an environmental temperature of 31 to 32°C. for 6 hours. On exposure to cold group A reacts with a hyperglycemia, B with hypoglycemia and D shows no significant change in blood sugar. The experiments show that cold acts on both vago-insulin and sympathetico-adrenal systems, the effect predominating on the latter. On exposure to heat group A reacts with a delayed hypoglycemia, B with hypoglycemia persisting during the whole period, C with a hyperglycemia and D with no significant change in blood sugar. Heat also acts on both vago-insulin and sympathetico-adrenal systems but the predominant effect is on the former. The significance of these reactions for homeostasis is discussed.

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WORK PERFORMANCE OF ADRENALECTOMIZED RATS TREATED WITH 11-DESOXYCORTICOSTERONE SODIUM PHOSPHATE AND 11 - DESOXY - 17 - HYDROXYCORTICOSTERONE

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The compound 11-desoxycorticosterone and its acetate are very weak in their effects on the work performance of adrenalectomized rats (3) as compared to corticosterone, 11-dehydrocorticosterone, 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone. The compound 11-desoxycorticosterone and its acetate are not soluble in water in therapeutically effective concentrations, whereas corticosterone and similar compounds having a favorable effect on work are more soluble in water. It was considered possible that the effect of these compounds on work might be determined by rate of absorption rather than by the chemical differences in structure. Reichstein and Euw (5) have prepared the sodium salt of 11-desoxycorticosterone phosphate which is soluble in water. In the present study it was found that this water soluble form of the hormone is also very weak in its effect on work.

A second question concerns the relative importance of carbon 11 and carbon 17 as a site for location of an oxygen atom with respect to the effect on work. Reichstein and Schindler (6) have prepared 11-desoxy-17-hydroxycorticosterone by partial synthesis. An examination of the acetate of this compound has shown that the presence of the hydroxyl at carbon 17 has no more effect on work than the very weakly active 11-desoxycorticosterone acetate.

METHODS. Male rats of the Sprague-Dawley strain which weighed approximately 180 grams were used in these experiments. The diet was Purina Dog Chow. Bilateral adrenalectomies were performed in one

[1] I wish to express my appreciation to Dr. J. J. Pfiffner, Parke, Davis and Co., Detroit, Michigan, who supplied the 17-hydroxy-11-dehydro-corticosterone; Dr. E. Oppenheimer of the Ciba Pharmaceutical Products Co., Summit, New Jersey, who supplied the 11-desoxy-corticosterone acetate and the 11-desoxy-corticosterone sodium phosphate; and to Prof. T. Reichstein, Basle, Switzerland, who supplied the 11-desoxy-17-hydroxycorticosterone acetate.

stage under ether anesthesia. Immediately following operation the animals were anesthetized with phenobarbital sodium. The gastrocnemius muscle was weighted with 100 grams and stimulated to contract three times per second until muscular responsiveness was lost or until the animal

TABLE 1

Work performance of adrenalectomized rats treated† with adrenal steroids*

DOSE‡	Corticosterone derivatives				
	17-HYDROXY-11-DEHYDRO (IN OIL)	17-HYDROXY-11-DESOXY- (ACETATE) (IN OIL)	11-DESOXY- (ACETATE) (IN OIL)	11-DESOXY-SODIUM PHOSPHATE	
				(In oil)	(In water)
mgm.					
0.25	9286		3897	2503	2719
	10894		3994	6494	3410
	13629		4039	3295	2087
	12369		4210	3786	
			4123	2512	
0.50	16467	4737	3923	3560	4174
	17816	2722	3009	4433	4641
	19320	4791	3710	4612	2350
	12235	3292	5187	5399	
			2527	2420	
1.00		3560	6623	7789	3999
		1946	7233	7138	3032
		6132	5246	3437	3698
		5664	6889	4789	3051
			5737		
2.00		9049	3027	4324	3663
		6022	5027	4663	3587
		4642	6444	1987	6435
		4406	2220	3625	3393
			8233		

* Work is expressed as total number of recorder revolutions. Each recorder revolution represents approximately 400 gram centimeters of work.

† The range in performance of 10 untreated animals was 2452–6995 recorder revolutions.

‡ The dose expressed above was administered at the beginning of stimulation and again six hours later in the animals which continued to work for this period of time.

had worked for 24 hours. Each animal received 5 cc. of water by subcutaneous injection at the beginning of stimulation and again six hours later in those animals which continued to work for this period of time. The test substances were also administered by subcutaneous injection at the beginning of stimulation and again six hours later in those animals

which had not already shown collapse. The details of the method have been described (1, 2).

EXPERIMENTS AND RESULTS. The work performance of adrenalectomized rats treated with 11-desoxycorticosterone sodium phosphate (in water and in sesame oil media) and with 17-hydroxy-11-desoxycorticosterone acetate was compared to that of untreated adrenalectomized rats and adrenalectomized rats treated with 17-hydroxy-11-dehydrocorticosterone and with 11-desoxycorticosterone acetate. The data on dosage and the values for work are given in table 1.

The work performance of the rats treated with 0.25 mgm. and 0.5 mgm. of 17-hydroxy-11-dehydrocorticosterone per dose was clearly enhanced above the work performance of untreated rats in every instance. Seven of the eight animals treated with this substance were still working at the end of the 24-hour test period whereas all of the remaining animals were "fatigued" before this time and showed values for work characteristic of untreated animals.

The relative activities of the adrenal steroids in respect to work and diabetogenic or anti-insulin activity are parallel. Ingle and Lukens (4) have found that the ability of the adrenalectomized rat to continue work is dependent in part upon the availability of glucose. The administration of 2 mgm. daily of 17-hydroxy-11-desoxycorticosterone to a partially depancreatized rat failed to induce a glycosuria but the administration of 1 mgm. daily of 17-hydroxy-11-dehydrocorticosterone caused the excretion of up to 2.0 grams daily of glucose. It is probable that the compound 17-hydroxy-11-desoxycorticosterone does not have a significant effect on carbohydrate metabolism.

CONCLUSIONS

The water soluble 11-desoxycorticosterone sodium phosphate is approximately as weak in its effect on the work performance of adrenalectomized rats as is the water insoluble 11-desoxycorticosterone acetate, thus demonstrating that the inactivity of 11-desoxycorticosterone in the work test is not due to failure of absorption.

The presence of oxygen at carbon 17 does not enhance the effect on work as does the presence of oxygen at carbon 11; for 17-hydroxy-11-dehydrocorticosterone is very active in this respect whereas 17-hydroxy-11-desoxycorticosterone has little if any effect.

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CREATININE-CREATINE EXCRETION IN SCHIZOPHRENICS

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Our present conceptions regarding the excretion of creatinine and creatine arise primarily from the observations of Folin (1905). His subjects were patients and workers living on a meat-free diet in a mental hospital. He found that the creatinine elimination from day to day was practically constant for the same individual, but varied for different individuals, and was entirely independent of the protein intake. The absence of creatine in the urine of adult males, its presence in the urine of children in the prepubertal period and of normal adult women has been generally accepted following further observations made by Folin and others.

However, it should be noted that Folin and Denis (1912) found some creatine in the urine of a 17-year-old boy. Later Light and Warren (1934) reported 19 years as the limiting age at which creatine fails to be normally excreted in the urine of males. In this connection the reports of Taylor and Chew (1936), Hobson (1939) and Dill and Horvath (1941) on the occurrence of creatine in the urine of adult males are particularly interesting. Of these workers only Taylor and Chew had their subjects on a meat-free diet. Dill and Horvath also noted, as had other observers, that creatinine elimination was not as constant as was previously assumed (Best and Taylor, 1937).

It seemed worth while to repeat these early observations on the same general type of patient as had been used by Folin, keeping in mind the sexual variations. The creatinine and creatine were the same substances as measured by Folin using identical methods, except that a photo-electric colorimeter was used instead of a visual colorimeter. Sixteen schizophrenics, eight males and eight females, were used as subjects. They are patients at the Metropolitan State Hospital, Waltham, Massachusetts. They had no disturbances, such as progressive muscular dystrophy and Graves' disease, in which creatine is frequently found. A brief protocol of the subjects is appended.

These patients were isolated and kept under constant supervision to insure the complete collection of urine. Twenty-four-hour collections of urine were obtained on two consecutive days during one or two successive weeks while the patients were on the regular hospital diet. They were

then placed on a meat-free diet consisting essentially of milk, bread, vegetables and fruit. Two twenty-four-hour collections of urine were made after the second day of the diet and again after twelve days. The subjects were then returned to their usual diets and urines were obtained after a period of at least one week had elapsed.

The freshly voided urine was preserved with thymol and kept in a refrigerator at about 4°C. Determinations of preformed creatinine and total creatinine by Folin's method, using the Evelyn photo-colorimeter, were made immediately on the completion of a collection. Preformed creatinine was determined colorimetrically after the addition of freshly made

	DIAGNOSIS	AGE	HEIGHT	WEIGHT
	Males			
				pounds
E. A.	D.P. Hebephrenic	52	5′ 6″	148
F. A.	D.P. Hebephrenic	42	5′ 6″	120
E. C.	D.P. Hebephrenic	37	5′ 2″	140
G. C.	D.P. Hebephrenic	41	5′ 6″	137
J. C.	D.P. Hebephrenic	31	5′10″	163
D. D.	D.P. Hebephrenic	37	5′10″	183
H. D.	D.P. Hebephrenic	37	5′ 9″	130
J. P.	D.P. Hebephrenic	34	5′ 6½″	144
	Females			
A. A.	D.P. Type undetermined	38	5′4″	169
D. A.	D.P. Paranoid	29	5′ ½″	99
A. B.	D.P. Hebephrenic	29	4′11½″	181
H. B.	D.P. Catatonic	38	5′6½″	109
C. C.	D.P. Hebephrenic	33	5′1½″	172
A. C.	D.P. Catatonic	37	5′7½″	112
E. L.	D.P. Catatonic	32	5′5½″	114
E. C.	D.P. Catatonic	29	5′4″	119

alkaline picrate to the fresh urine. For total creatinine a sample was autoclaved with one normal HCl at a temperature of 120° for 30 minutes and to an aliquot alkaline picrate was also added, as for creatinine. Creatine, expressed in terms of creatinine, was obtained by difference. Total nitrogen determinations were made by the Kjeldahl method.

Results. The data are presented separately for male and female subjects. The average nitrogen excretions, which are shown graphically in figure 1, require no comment. On the meat-free diet the nitrogen decreased and on resumption of the hospital diet tended to return to previous levels. As noted by others, there is a smaller excretion of creatinine in the female. On the usual diet, the average creatinine values of 0.92 and 0.93 gram

agree closely to the 0.90 gram average found by Tracy and Clark (1914) in their study of 26 normal and well-developed females. There was a fall to 0.74 gram while on the meat-free diet. The excretion of creatinine in the males did not change markedly as a result of diet but did increase in the period following the diet.

There is considerable variation in the amount of creatinine excreted on different days for both sexes (tables 1 and 2). For example, a male, J. P.,

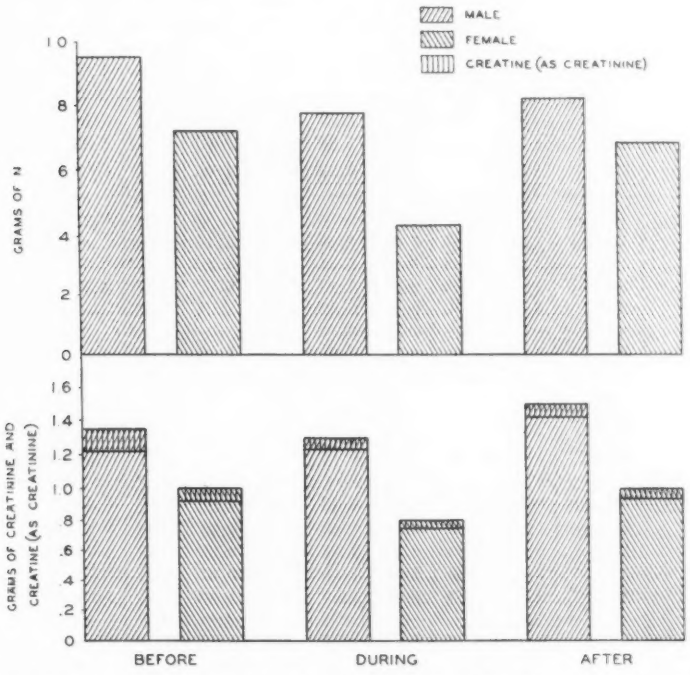

Fig. 1. Averages of twenty-four-hour excretions of creatinine and of creatine (as creatinine) and nitrogen in eight male and eight female schizophrenics before, during and after a meat-free diet.

on one day previous to the dietary period excreted 0.76 gram while after the diet 1.52 grams were excreted, the urinary volume being identical. Another male, E. A., excreted 1.56 grams before and 1.07 grams after the diet, again with almost identical urinary volumes. Similar examples of variability in creatinine excretion were observed among the females. As McLaughlin and Blunt (1923–1924) have stated, "The constancy of creatinine output, daily or hourly, is a relative term." The daily excretion for

TABLE 1

Twenty-four hour excretions of urinary nitrogen, creatinine and creatine in female schizophrenics before, during and after a meat-free diet

SUBJECT	PERIOD	VOLUME		TOTAL N		TOTAL CREATININE		CREATININE		CREATINE	
		I	II	I	II	I	II	I	II	I	II
		l.	*l.*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
A. C.	Before	1.67	1.16	7.02	9.62	1.11	1.12	1.07	1.02	0.04	0.10
	Before	0.52	1.02	8.92		0.72	1.42	0.66	1.21	0.06	0.21
	Diet	1.49	0.95	4.52	6.39	0.66	1.12	0.63	1.10	0.03	0.02
	Diet	1.51	1.14	5.31	3.74	0.90	0.75	0.84	0.68	0.06	0.07
	After	2.55	1.14	10.85	13.01	1.68	0.88	1.66	0.87	0.02	0.01
D. A.	Before	1.25	1.16	4.73	9.62	0.60	1.43	0.55	1.26	0.05	0.17
	Before	1.08	0.89	5.06	7.32	0.44	0.99	0.41	0.84	0.03	0.05
	Diet	1.00	0.84	3.14	3.92	0.48	0.74	0.41	0.69	0.07	0.05
	Diet	1.40	1.06	3.72	3.21	0.62	0.60	0.55	0.56	0.07	0.04
	After	1.60	1.02	4.66	5.32	0.59	1.09	0.56	0.98	0.03	0.09
E. L.	Before	0.73	1.38	7.75	6.78	1.09	0.98	1.01	0.83	0.08	0.15
	Before	1.38	0.80	6.68	4.33	1.12	1.19	1.09	1.10	0.03	0.09
	Diet	1.70	1.44	5.42	5.04	0.83	0.70	0.73	0.68	0.10	0.02
	Diet	1.09	0.65	2.99	3.20	0.41	0.61	0.87	0.57	0.04	0.04
	After	1.24	0.76	5.18	5.02	0.87	1.04	0.87	0.97	0.00	0.07
E. C.	Before	1.55	1.54	6.03	6.84	1.10	1.08	1.02	0.97	0.08	0.11
	Before	0.70	1.00	4.98	7.54	0.90	1.21	0.79	0.88	0.11	0.33
	Diet	1.45	0.78	4.60	3.52	0.75	0.73	0.70	0.65	0.05	0.08
	Diet	1.52	1.01	3.10	3.80	1.18	0.45	1.11	0.42	0.07	0.04
	After	1.72	1.20	4.38	5.72	0.83	1.03	0.77	0.91	0.06	0.12
H. B.	Before	0.91	0.74	9.06	6.43	1.11	1.09	1.07	1.02	0.04	0.07
	Before	1.60	1.51	4.35	4.37	1.09	0.88	1.02	0.84	0.07	0.04
	Diet	0.80	0.84	2.97	3.40	0.50	0.82	0.43	0.78	0.07	0.04
	Diet	0.42	0.31	2.69	2.45	0.55	0.81	0.51	0.70	0.04	0.11
	After	0.97	0.84	5.46	4.33	0.60	1.46	0.56	1.29	0.04	0.17
C. C.	Before	0.42	0.72	3.05	4.71	0.79	1.18	0.73	1.15	0.06	0.03
	Before	0.86	0.60	5.07	3.61	0.69	0.53	0.65	0.48	0.04	0.05
	Diet	0.79	0.53	3.25	2.88	0.55	0.76	0.47	0.71	0.08	0.05
	Diet	1.58	0.45	6.16	3.20	1.17	0.64	1.03	0.62	0.14	0.02
	After	0.74	1.32	3.14		0.61	0.67	0.59	0.65	0.02	0.02
A. B.	Before	1.51	0.70	8.91	5.57	1.18	0.93	1.15	0.89	0.03	0.04
	Before	0.72	0.69	5.60	5.73	0.74	0.84	0.66	0.78	0.08	0.06
	Diet	1.84	0.70	5.91	4.10	0.92	0.87	0.85	0.80	0.07	0.07
	Diet	1.86	1.76	6.53	6.12	1.10	1.14	1.03	1.06	0.07	0.08
	After	1.22	1.39	4.57	9.11	0.89	1.61	0.84	1.54	0.05	0.07
A. A.	Before	1.62	1.02	8.66	10.20	1.44	1.56	1.38	1.48	0.06	0.08
	Before	0.63	1.44		9.03	0.91	1.59	0.79	1.59	0.12	0.00
	Diet	1.83	0.65	7.88	4.23	1.03	0.80	0.92	0.72	0.11	0.08
	Diet	1.05	0.94	3.43	4.50	0.60	0.94	0.54	0.86	0.06	0.08
	After	1.71	1.27	9.96	12.34	0.86	1.03	0.81	0.95	0.06	0.08

TABLE 2

Twenty-four hour excretions of urinary nitrogen, creatinine and creatine in male schizophrenics before, during and after a meat-free diet

SUBJECT	PERIOD	VOLUME		TOTAL N		TOTAL CREATININE		CREATININE		CREATINE	
		I	II	I	II	I	II	I	II	I	II
		l.	*l.*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
J. P.	Before	0.48	0.74	5.79	9.48	0.78	1.39	0.76	1.36	0.02	0.03
	Diet	1.10	1.38	7.84	7.81	1.31	1.23	1.25	1.16	0.06	0.07
	Diet	0.44	1.47	5.00	5.27	0.61	2.32	0.59	2.25	0.02	0.07
	After	0.48	0.74	6.82	11.87	1.58	1.57	1.52	1.54	0.06	0.03
J. C.	Before	1.18	0.73	9.80	7.65	1.91	1.34	1.88	1.28	0.03	0.06
	Diet	1.24	0.92	6.78	6.64	1.98	1.21	1.98	1.18	0.00	0.03
	Diet	1.02	1.06	5.72	8.71	1.13	1.55	1.12	1.54	0.01	0.01
	After	1.70	0.90	5.98	7.86	1.53	1.67	1.53	1.65	0.00	0.02
D. D.	Before	1.07	0.98	8.29	6.74	1.24	1.02	1.18	0.99	0.06	0.03
	Diet	0.78	0.55	7.26	5.03	0.88	0.91	0.88	0.89	0.00	0.02
	Diet	0.80	0.86	9.67	6.66	1.54	1.34	1.50	1.32	0.04	0.02
	After	1.36	0.98	6.62	7.37	1.42	1.57	1.39	1.52	0.03	0.05
E. C.	Before	1.64		14.50		1.20		1.13		0.07	
	Before	0.84	1.06	11.38	10.11	1.34	1.46	1.19	1.37	0.15	0.09
	Diet	1.67	1.40	7.71	9.46	1.27	1.65	1.27	1.51	0.00	0.14
	Diet	1.33	1.63	11.72	10.46	1.85	1.68	1.78	1.66	0.07	0.02
	After	1.44	1.18	10.78	10.43	1.56	1.77	1.50	1.69	0.06	0.08
E. A.	Before	1.34		13.60		1.65		1.56		0.09	
	Before	1.00	1.30	10.07	10.59	1.61	1.39	1.27	1.26	0.34	0.13
	Diet	1.08	1.24	4.68	9.30	0.76	1.28	0.75	1.24	0.01	0.04
	Diet	1.03	0.93	7.11	9.60	1.13	1.14	1.04	1.14	0.09	0.00
	After	1.35	1.13	6.77	7.77	1.11	1.41	1.07	1.35	0.04	0.06
F. A.	Before	1.21		14.10		1.91		1.32		0.62	
	Before	0.64	0.68	7.97	8.50	1.32	1.18	0.86	0.89	0.46	0.29
	Diet	1.20	1.22	7.95	10.11	1.15	1.35	0.97	1.16	0.18	0.19
	Diet	0.99	1.22	7.12	9.51	1.37	1.57	1.24	1.32	0.13	0.25
	After	1.30	0.89	7.93	8.47	1.39	1.65	1.20	1.34	0.19	0.21
G. C.	Before	1.27		12.03		1.44		1.40		0.04	
	Before	0.96	1.24	7.06	9.82	0.99	1.33	0.97	1.29	0.02	0.04
	Diet	1.20	1.08	7.03	6.63	1.28	0.89	1.26	0.86	0.02	0.01
	Diet	0.90	1.05	6.30	8.00	1.04	1.25	1.03	1.21	0.01	0.04
	After	0.96	0.92	4.76	7.23	0.88	1.20	0.85	1.15	0.03	0.05
H. D.	Before	0.67		8.00		1.36		1.32		0.04	
	Before	1.10	1.26	9.37	5.35	1.55	0.90	1.41	0.82	0.14	0.08
	Diet	1.14	1.13	6.84	8.87	1.47	1.50	1.40	1.39	0.07	0.11
	Diet	0.60	0.85	3.29	3.78	0.71	0.73	0.67	0.70	0.04	0.03
	After	0.98	0.78	12.53	8.47	2.10	1.52	2.05	1.43	0.05	0.09

a single individual varies within limits, frequently differing more than 25 per cent.

From the data of Folin (1905) on ten series of observations the following variability was noted:

	1	2	3	4	5	6	7	8	9	10
Mean	1.17	1.49	1.55	1.14	1.36	1.56	1.81	1.13	1.34	1.37
Extremes	1.28–1.02	1.66–1.33	1.65–1.36	1.22–1.05	1.48–1.28	1.77–1.32	1.90–1.66	1.38–1.01	1.51–1.20	1.62–1.23
Range in per cent of mean.	22	21	18	15	15	28	13	32	22	28

Similar variations were also found in the data of Benedict and Myers (1907) in their study of the creatinine excretion of female patients. Dill and Horvath, among others, have also noted a variation in creatinine excretion. The use of creatinine excretion for testing the completeness of a twenty-four-hour urinary output is a crude procedure but is one in which many workers continue to place unwarranted confidence.

We can verify the statement (Hodgson and Lewis, 1928–1929) that creatine is excreted by adult females whether on their usual or on a meat-free diet (table 1). If for purposes of argument we assume, as had Hodgson and Lewis, that any value for creatine less than 0.02 gram be ignored then it will be noted that in 80 observations there was an elimination of creatine in 77 or 96 per cent. Twenty-nine of these were found in the 32 observations obtained when they were on the meat-free diet and an additional 11 in the 16 observations during the period following this diet. But the male subjects (table 2) also showed a creatinuria. Of a total of 69 observations, creatine was found 51 times (86 per cent). Eighteen of these were in the 32 urines examined during the duration of the meat-free diet. Every one of the eight subjects while on the diet had creatine in his urine in at least one of his four samples obtained during this time. Following resumption of the hospital diet, creatine was found in 14 of the 16 urines examined. One of these males, F. A., excreted as much as 0.250 gram per 24 hours while on the meat-free diet. Previous to this period of dietary control he had in one instance excreted 0.620 gram, which approaches the values (0.80 gram) found by Dill and Horvath in a normal male laboratory technician.

The average of all observations on both male and female subjects is shown in figure 1. Males and females excreted approximately identical amounts of creatine during their dietary regimes. During the period of meat-free diet they both excreted approximately 0.06 gram. Even if we eliminate from our male averages the values obtained in the case of F. A., there is still a definite elimination of creatine (0.034 gram).

The data presented in this study confirm the belief that it is common for adult schizophrenic males to eliminate creatine and that it is not an age or sex limited function. The possibility of creatine elimination cannot be dismissed as easily as it has been in the past. Furthermore, the constancy of the daily excretion of creatinine in a single subject is assumed only relatively constant as compared to the inconstancy of the elimination of some of the other urinary constituents, such as urea.

SUMMARY

Creatine is present in the urine of adult schizophrenic males. When they were placed on a meat-free diet, creatine was still observed in 86 per cent of the urines examined. In common with other investigators we found creatine in the urine of female schizophrenics at all times.

In both sexes creatinine excretion lacks the constancy attributed to it by Folin and others. It varies considerably and differs from day to day more than 20 per cent.

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PERIPHERAL VASCULAR RESPONSES IN MAN DURING DIGESTION[1, 2]

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The influence of the process of digestion on the circulatory system is well recognized. An acceleration of the pulse rate (1, 2, 3), an increase in pulse pressure (1, 2, 3) and an augmentation in cardiac output (1, 3, 4) occur shortly after eating. The question arises as to whether or not the rate of blood flow to the periphery is similarly affected. Most of the evidence in this respect is of an indirect nature, consisting of studies of skin temperature (2, 5) and circulation time (6, 7). The only direct determination of the effect of digestion on the peripheral circulation is the work of Herrick and her associates (8) on dogs. By using a modification of the Rein thermostromuhr they found that the blood flow through the femoral and carotid arteries increased to approximately double that in the fasting animal.

In view of the paucity of information concerning the postprandial peripheral vascular responses in man, the present study was undertaken to determine the effect of a predominantly carbohydrate or protein meal upon the total blood flow through the extremities, using the venous occlusion plethysmographic method.

METHOD. Seventeen experiments were conducted on eight normal subjects (7 males and 1 female) in the postabsorptive state. The technique employed was similar to that previously described (9), the readings being expressed in cubic centimeters per minute per 100 cc. limb volume. In all, the hand was studied 15 times, the forearm 10, and the leg 6 times. First, a control level of blood flow was obtained by averaging the results of 10 to 15 determinations taken over a period of one-half hour, and then a weighed diet of approximately 400 calories of mainly protein or carbohydrate was fed to the subject over a period of 25 to 30 minutes. The protein meal consisted essentially of lean meat, cottage cheese, egg white and gelatine, while the carbohydrate meal included vegetables, sweetened stewed and raw fruits, and sweetened fruit juice. In order to determine whether or

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[2] Presented before the American Physiological Society, April 1941.

not the fluid content of the diet would affect the results, some of the meals were made up predominantly of liquids, while others contained only a minimum of water. In a number of experiments, a larger meal containing 600 to 800 calories was given. Following the ingestion of food, blood flow readings were made every few minutes for the subsequent 3 to 4 hours. In some instances the same individual was utilized to study separately the effect of both carbohydrate and protein meals.

Besides blood flow studies, the pulse rate, blood pressure and rate of oxygen consumption were also noted anteprandially and at least once every half-hour in the period after eating. All experiments were conducted under physiological conditions, with the room temperature varying be-

TABLE 1

Typical responses to the ingestion of carbohydrate

TIME	BLOOD FLOW			CALORIES	BLOOD PRESSURE	PULSE RATE	TIME	BLOOD FLOW			CALORIES	BLOOD PRESSURE	PULSE RATE
	Hand	Forearm	Leg					Hand	Forearm	Leg			
	Subject 7							Subject 5b					
hours				*sq.m./hr.*			*hours*				*sq.m./hr.*		
Control	10.0	1.5		36.0	106/70	63	Control			1.5	34.7	116/84	72
½	9.3	1.5		40.7	108/66	66	½			1.4	39.7	116/84	78
1	11.4	1.6		40.7	104/66	70	1			1.7	36.9	116/84	77
1½	11.2	1.6		40.7	106/68	76	1½			1.7	38.0	120/84	80
2	11.8	1.8		42.1	104/68	62	2			1.7	38.5	122/86	78
2½	11.2	1.5		40.7	100/68	63	2½			1.6	38.0	118/84	78
3	11.7	1.5		38.1	104/70	60	3			1.7	35.8	116/86	72
3½	11.4	1.3		39.4	102/70	58							

Blood flow expressed in cubic centimeters per minute per 100 cc. limb volume.

tween 25° to 27°C. and the temperature of the water in the plethysmograph being maintained at 32°C.

Results. Since the vascular beds in the forearm and leg for the most part have similar physiological responses, the data obtained from these portions of the extremities will be treated together. The blood vessels in the hand, however, react differently from those in the former two (10), and hence the findings in this site will be dealt with separately.

Effect of a carbohydrate meal. The circulation in the forearm and leg during the 2½ to 3 hour postprandial period was relatively unaffected by the ingestion of a carbohydrate meal, regardless of its water content (table 1). Generally, the hand flow also remained unchanged (fig. 1), although in two cases a definite decrease was noted in the first hour (fig. 2). In one instance an increase was present within ½ hour after eating, but in

this case the control blood flow in the hand was below normal levels. The same subject, on two other occasions, showed no significant alteration in hand circulation.

Generally the pulse pressure increased an average of 6 mm. Hg almost immediately after eating, chiefly as a result of a rise in systolic blood pressure. It remained increased for two hours and then tended to return toward the basal value (figs. 1 and 2). The pulse accelerated an average of 9 beats per minute in the first 1½ hours and then slowed toward the control

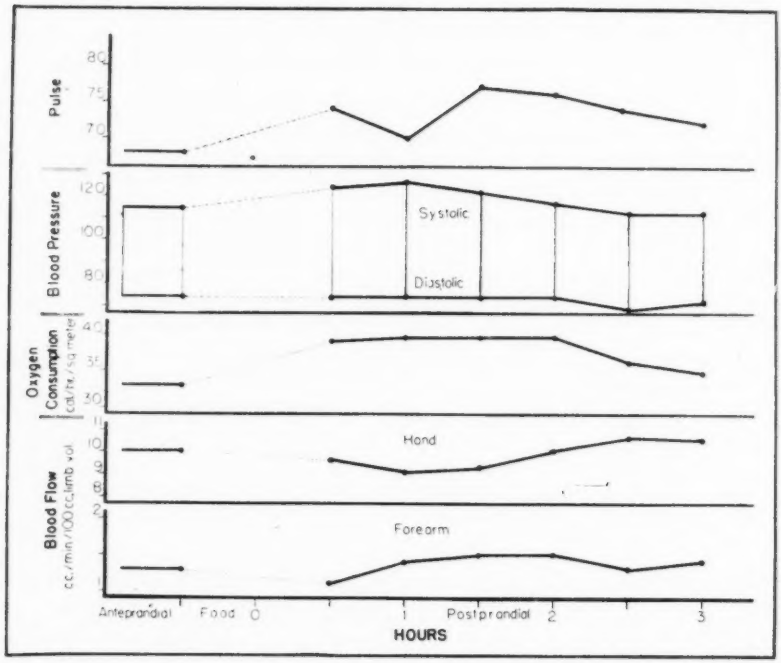

Fig. 1. Effect of ingestion of 550 calories of carbohydrate. Subject 2e (not included in table 1).

rate within 2½ hours postprandially. The rate of oxygen consumption rapidly rose during the first 30 minutes, to reach a peak within 1½ hours postprandially, and gradually decreased thereafter. The maximal average increase elicited by the carbohydrate meal was 6.3 calories per square meter per hour (an average percentage increase of 20).

Thus, the pulse pressure, pulse rate, and oxygen consumption were increased during the digestion of a carbohydrate meal, without any concomitant significant augmentation in the circulation taking place in the forearm, leg, and hand.

Effect of a protein meal. After the ingestion of a protein meal, little change was observed in peripheral blood flow in the first 1½ hours (table 2). During this interval, the pulse accelerated an average of 11 beats per minute, and the pulse pressure was widened an average of 11 mm. Hg. An increase in the rate of metabolism, averaging 6 calories per square meter per hour, occurred in the first 30 minutes after eating, reached a maximum of 10 calories in 90 to 150 minutes (an average percentage increase of 30),

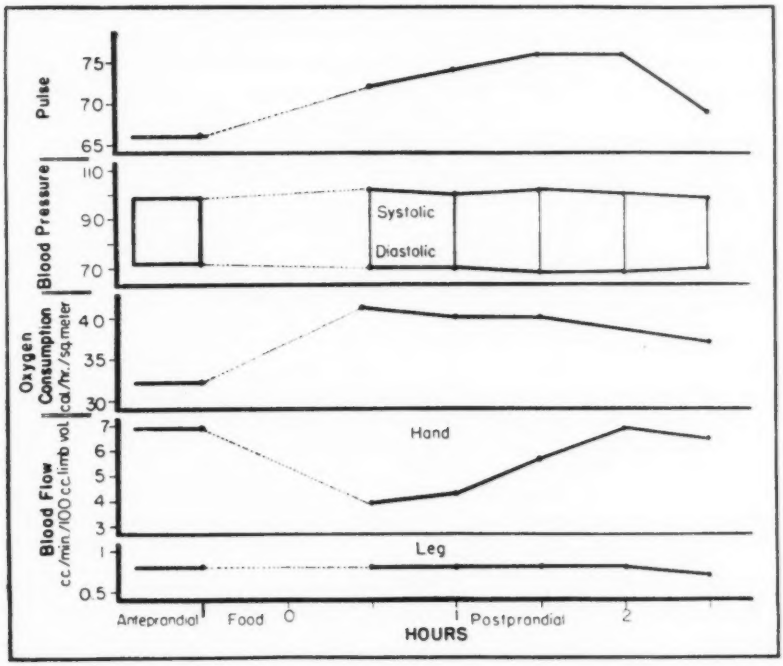

Fig. 2. Effect of ingestion of 400 calories of carbohydrate. Subject 1 c (not included in table 1).

to continue so until the end of the experiment. After the lapse of 1 to 1½ hours, an increase in blood flow became manifest in the hand, the highest level occurring in 2 to 2½ hours. The circulation remained enhanced during the rest of the experiment (fig. 3). The forearm and leg did not show any augmentation in arterial inflow until 1½ to 2½ hours had elapsed. At this time an increase was noted, which reached its highest level in 2½ to 3 hours, to continue so for the remainder of the procedure (fig. 3). In one subject the circulation in the hand increased 60 per cent,

while the forearm flow did not change, possibly because a postprandial period of only 2 hours had elapsed before the experiment was terminated. When the experiment was repeated and continued for a longer interval, an increase in forearm flow of 50 per cent was noted 3 hours after eating (table 2, subject 3b).

Thus, during the first hour after a protein meal, no alteration was observed in blood flow, despite significant changes in the other factors studied. Only after $1\frac{1}{2}$ to 3 hours did an augmentation in peripheral circulation appear, at which time the pulse rate, pulse pressure and rate of oxygen consumption had already reached a plateau or were diminishing.

Discussion. The changes occurring in the cardiovascular system after eating have been studied by several investigators (1, 2, 3). Grollman

TABLE 2

Typical responses to the ingestion of protein

TIME	BLOOD FLOW			CALORIES	BLOOD PRESSURE	PULSE RATE	TIME	BLOOD FLOW			CALORIES	BLOOD PRESSURE	PULSE RATE
	Hand	Forearm	Leg					Hand	Forearm	Leg			
	Subject 2a							Subject 3b					
hours				*sq.m./hr.*			*hours*				*sq.m./hr.*		
Control	7.7		1.3	31.1	98/64	64	Control	7.6	0.8		32.7	118/82	78
$\frac{1}{2}$	7.5		1.2	35.6	100/64	72	$\frac{1}{2}$	6.3	0.8		42.6	126/84	82
1	7.4		1.1	34.7	96/64	68	1	6.6	0.8		40.2	132/78	88
$1\frac{1}{2}$	8.3		1.4	35.7	94/54	70	$1\frac{1}{2}$	7.6	0.9		44.0	128/80	86
2	9.9		1.5	36.4	94/54	69	2	11.2	0.9		41.3	128/80	88
$2\frac{1}{2}$	10.7		1.7	35.8	94/54		$2\frac{1}{2}$	10.4	0.8		42.6	128/80	88
3	9.6		2.0	38.0	92/54	69	3	10.0	1.2		44.0	126/80	88
							$3\frac{1}{2}$	9.6	1.2		44.0	124/78	80

Blood flow expressed in cubic centimeters per minute per 100 cc. limb volume.

(3), using a mixed diet, found that the cardiac output rose from a basal level of 3.43 liters per minute to 4.72 liters within one quarter-hour after ingestion of food, and remained elevated during the following three hours. He indicated that this rather abrupt change might be due to a viscerocardiac reflex initiated from the digestive tract. Apéria and Carlens (4) fed carbohydrates and proteins separately and found that different types of cardiovascular responses were elicited by these substances. The ingestion of sucrose resulted in a rapid transient rise in metabolism and cardiac output, and a gradual decline to basal levels within $1\frac{1}{2}$ to $2\frac{1}{2}$ hours after eating. With the administration of protein, the cardiac output was likewise increased, but the major response occurred $2\frac{1}{2}$ to $5\frac{1}{2}$ hours postprandially, with a return to the control level taking place in $6\frac{1}{2}$ hours.

In view of this alteration in cardiac output, an augmentation in blood flow through the systemic circulation would be expected. It is generally recognized that such a change does take place in the splanchnic region, but the evidence as to the effect of the ingestion of food upon peripheral blood flow is scanty. The few reported investigations suggest that the postprandial period is associated with an increase in circulation through the extremities. McCracken and his associates (6), using the ionization method in the dog, found a decrease of from 12.9 to 45.4 per cent in the

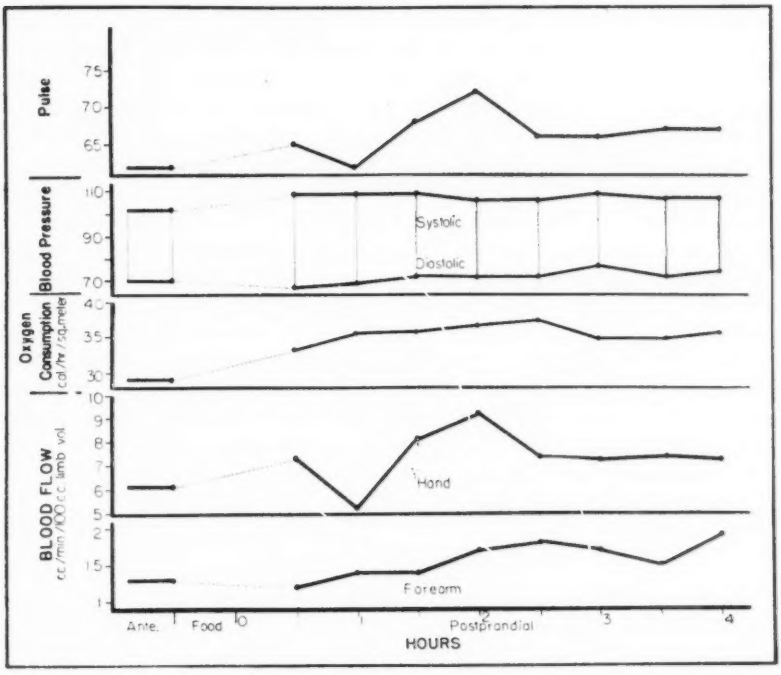

Fig. 3. Effect of ingestion of 600 calories of protein. Subject 2b (not included in table 2).

circulation time from the internal jugular vein to the femoral artery. Burton and Murlin (5) reported an increase in skin temperature in man which commenced 20 minutes postprandially, reached a maximum in the second hour, and then fell in the third. Aside from these indirect methods, Herrick and her co-workers (8) used the thermostromuhr and determined the rate of blood flow in the femoral and carotid arteries of the dog after a protein or carbohydrate diet. They found that the circulation increased approximately 70 per cent above the control level,

beginning within 15 minutes after eating and lasting 1½ to 2½ hours. The only difference noted between the effects of protein and carbohydrate meals was the rapidity with which the peak of the response to the latter foodstuff occurred.

The data obtained in the present investigation are not entirely in accord with the findings of the above authors. That digestion of the food had taken place in each of our experiments can be inferred from the significant alteration observed in oxygen consumption. Further, the finding of an increased pulse rate and pulse pressure suggests that an augmented cardiac output had probably also occurred. In spite of these changes, as already stated, no significant increase in the rate of blood flow to the hand, forearm and leg was noted following carbohydrate ingestion. It would appear, therefore, that under such conditions the increased minute volume output of the heart was adequately compensated for by either splanchnic vasodilatation alone, by peripheral vasoconstriction of a degree sufficient to vitiate the effect of the increased cardiac output, or by a combination of both factors. In respect to protein ingestion, the increased peripheral blood flow was first observed only after the changes in cardiac output presumably had been present for some time. A direct causal relationship between these two factors cannot therefore be assumed, unless it is premised that either an already existing peripheral vasoconstriction, initiated at the onset of digestion, is removed 2 to 3 hours afterwards, or a shunting of blood from the splanchnic vessels to the periphery takes place in this period. The latter view does not appear reasonable, since digestive and absorptive processes are still at a peak at this time. Another factor to be considered is the initiation of an active peripheral vasodilatation, possibly as a result of some product of protein digestion.

SUMMARY AND CONCLUSIONS

1. The peripheral vascular responses to the ingestion of predominantly carbohydrate or protein meals were studied in a series of 8 normal subjects by means of the venous occlusion plethysmographic method.

2. A carbohydrate meal elicited no significant changes in the rate of peripheral blood flow in the hand, forearm, and leg. At the same time, however, definite increases in the rate of oxygen consumption, in pulse rate, and in pulse pressure were observed.

3. With a protein meal, there was no change in peripheral circulation for the first 1 to ½ hour postprandial period, and then generally the rate of blood flow began to increase, first in the hand and later in the forearm and leg, to remain elevated until the end of the experiment. The changes in oxygen consumption, pulse rate and pulse pressure, were similar to those observed with carbohydrate, except that they were of greater magnitude.

We wish to express our appreciation to Miss Marian Peterson and Miss Beatrice Rubin of the Department of Dietetics of the Jewish Hospital for their assistance in preparing the meals used in the study.

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REFLEXOGENIC COMPONENTS OF BREATHING[1, 2]

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Although these experiments deal primarily with the reflexogenic components of breathing it is desirable to keep in mind that two great forces drive the respiratory act—the inherent physico-chemical forces arising directly in the automatically discharging respiratory neurones, and the physico-chemical forces set up in these same cells by the impingement of nerve impulses coming from the outlying receptors. These two driving forces (direct and indirect) have much in common. Not only will a steady central or a steady reflex chemical drive elicit rhythmic respiratory activity, but each drive of itself is capable of creating a similar pattern of discharge (Gesell, Lapides and Levin, 1940; Brown, Atkinson and Gesell, 1939). These facts combined with the actual demonstrable addition of centrogenic and reflexogenic drives to one another are strong evidence for the existence of one common mechanism of nerve cell activation. Such views, as we shall try to show, allow an elementary yet broad interpretation of the respiratory act.

METHOD. The experimental procedures in these studies were extremely simple. Anesthetized dogs, connected with a delicately responding Hutchinson spirometer, were subjected to varying types and combinations of sensory stimulation and the effects of such stimulation were recorded upon smoked paper. Unless otherwise specified on the original tracings, upward movement indicates a filling of the lungs and downward movement the reverse. "Chemical reflexogenic drive", or more correctly its equivalent, was experimentally provided by faradic stimulation of Hering's nerve, usually after double vagal section. Stimulation of cutaneo-sensory nerves, important highways of impulses for the perception of pain, permitted experimental modification of pain drive. Central faradic stimulation of the cervical vagus offered a means of experimental modification of the periodic proprioceptive drives, such as those arising in the stretch receptors of the lungs. The nearly comparable action of central stimulation of the superior laryngeal nerve was also studied in some detail. Although the nerves which we have selected for investigation may be regarded as serving spe-

[1] Preliminary report: This Journal, Proc. **129**: P373, 1940.

[2] These experiments were supported by a grant from the Rockefeller Foundation.

cialized sensory functions in relation to respiration, the admixture of several types of sensory fibers in each of them must not be overlooked. It is with this in mind that we have employed the adequate chemical stimulation of the chemoceptors by intravenous injection of cyanide for comparison with the artificial electrical stimulation of Hering's nerve. For similar reasons the effects of stretching of the lungs by inflation were compared with faradic stimulation of the vagus. As we shall see, unselective artificial stimulation of mixed nerves, rather than confusing the issue, has seemed to clarify it and establish a unity and simplicity of principles. A distinct advantage of artificial electrical stimulation is the opportunity afforded of providing afferent signals in either steady or periodic streams. Thus the chemoceptor and nociceptor signals which normally impinge upon the center in a steady stream could be provided in periodic groups and conversely the periodically impinging proprioceptive signals could be supplied in a steady, continuing stream. Such artificial alterations revealed a singleness of action of excitatory signals regardless of their origin.

RESULTS. *Superior laryngeal nerve.* The most common result of faradic stimulation of this nerve is a purely expiratory response (see fig. 1) in which the lungs are brought to the expiratory position and rhythmic inspirations held in check. The degree of expiratory activity varies considerably with intensity of stimulation and with the individual. In figure 1 it is relatively weak yet definitely present, for as stimulation continues the lungs constrict below the normal expiratory volume. More striking examples of active contraction of the expiratory muscles are seen in figures 19 and 20 where the lung volume is promptly constricted on reflex stimulation. Results such as these give adequate reason for classifying the superior laryngeal nerve, in agreement with earlier workers (see Rosenthal, 1862), as primarily expiratory in action. Less commonly this predominant expiratory action is ushered in by a short inspiratory contraction thus indicating the existence of an inspiratory action as well. Such contractions are faintly visible in figure 23 in the first few stimulations of the superior laryngeal nerve; but since they are more readily elicited by vagal stimulation they will be described in greater detail under the next heading.

Cervical vagus nerve. The variability of response to faradic stimulation of the central end of the vagus nerve has been a perplexing problem of long standing. Rosenthal (1862) ascribed the expiratory action reported by his contemporaries to the effects of escape current reaching the superior laryngeal nerve, for when such possibilities were avoided he found only an inspiratory response. Later Hering and Breuer (1868) again insisted on an expiratory action of the vagi. Gad (1880) in turn rejected the classical interpretation of Hering and Breuer and postulated a purely inspiratory inhibitory action instead. Head (1889), Adrian (1933), Hillenbrand and Boyd (1936), Boyd and Maaske (1939) and many others described a similar

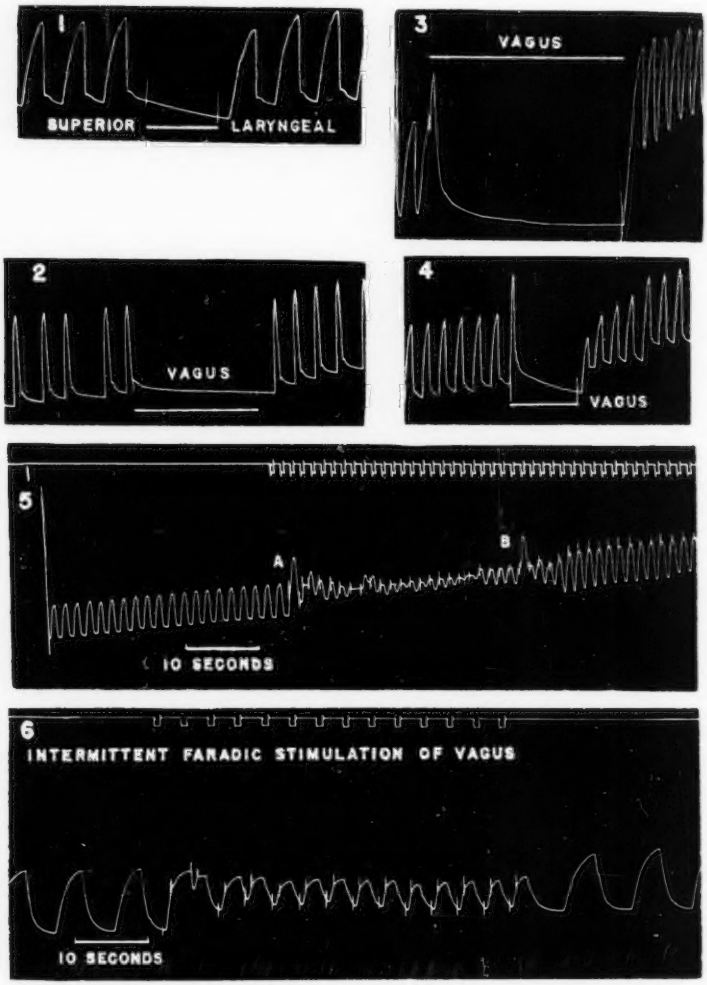


Fig. 1. Respiratory response to faradic stimulation of the superior laryngeal nerve of the dog. Breathing is recorded with a Hutchinson spirometer in circuit with a rebreathing tank. Upstroke represents inspiration of air and downstroke expiration. Both vagus nerves are cut in this observation and all others with the exception of figures 13 and 15. Note the increasing expiratory activity in the so-called "expiratory pause."

Fig. 2. Respiratory response to faradic stimulation of the cervical vagus nerve beginning during the expiratory phase of breathing. The results are comparable in every respect to those of figure 1.

Fig. 3. Respiratory response to faradic stimulation of the vagus nerve beginning

inspiratory inhibitory action. More recently a dual excitatory action has been proposed, the evidence for which appears mainly in preliminary communications (Gesell, 1939a, b, 1940a, b, 1939c, and 1940c; Gesell and Moyer, 1941; Worzniak and Gesell, 1939).

Figure 2 shows the commonly described effects of faradic stimulation of the central end of the cervical vagus nerve. They differ in no important respects from those already described for the superior laryngeal nerve in figure 1. Due to the cessation of rhythmic inspiration, the vagus has been commonly regarded as an inspiratory inhibitory nerve. But such interpretation overlooks expiratory activity and its reciprocal inhibitory action upon the inspiratory half-center for it will be noted that the expiratory volume diminishes as stimulation continues. This increasing expiratory activity, like that in figure 1, is no doubt the result of temporal summation in the expiratory half center. Stronger expiratory action is illustrated in figures 21 and 26. But when stimulation begins during the inspiratory phase of breathing instead of the expiratory phase (see figs. 3 and 4), the results are often importantly modified. In figure 4, where stimulation occurs at the beginning of the inspiratory act, the depth of inspiration is markedly increased. Where stimulation occurs toward the close of the inspiratory phase a second inspiration is superimposed (see fig. 3). The shortness of the added inspiratory activity in both instances is of the greatest interest for once the extra inspiratory activity has subsided rhythmicity disappears, as in figures 1 and 2, and gives way to a purely expiratory effect. The fact that expiration continues over a longer period than the introductory inspiratory activity indicates that the expiratory com-

in the last stages of the inspiratory phase, showing a powerful additional inspiratory contraction. After this added inspiratory response has subsided the results are comparable to those in figure 2.

Fig. 4. A heightened inspiratory response produced by faradic stimulation of the vagus nerve at the beginning of the inspiratory phase of breathing. This augmented inspiratory activity promptly gives way to a sustained expiratory contraction of supernormal strength.

Fig. 5. Variable response to a uniform frequency of interrupted faradic stimulation of the vagus nerve resulting from a failure of the respiratory rhythm to conform with the artificial rhythm of stimulation. At the end of the record where adjustment finally occurs expiration is uniformly augmented.

Fig. 6. A predominantly inspiratory response to an intermittently interrupted faradic stimulation of the vagus nerve. This type of stimulation is provided by a specially devised rotary interrupter constructed to stimulate one or two nerves and to control frequency of interruption and duration of the periods of stimulation and no stimulation, and the time relation of the stimulation of one nerve to the other. The frequency, duration and strength of stimulation remain unaltered in this figure. Respiration adjusts itself to respond with inspiration to each stimulation. Due to the short duration of stimulation and the long duration of inspiration the stimulation can exert no expiratory action.

ponent of vagal stimulation is more powerful than the inspiratory component.

It is, therefore, most significant that artificial inflation of the lungs which presumably provides an adequate and selective stimulation of the pulmonary stretch receptors may also produce a selective reinforcement of either the inspiratory or expiratory act (Worzniak and Gesell, 1939; Gesell and Moyer, 1941). Dual excitation under such physiological conditions warrants the belief that each stretch receptor synapses at both the inspiratory and expiratory half-centers and therefore is potentially capable of stimulating either half. Since stimuli must be impinging simultaneously upon both half-centers it becomes imperative that they discharge only one half-center at a time—the inspiratory half-center during the inspiratory phase and the expiratory half-center during the expiratory phase. We believe this alternate activity depends upon the "principle of the precedence of stimulation"; that the impulses impinging upon the expiratory half-center during the inspiratory phase of breathing are held in abeyance by the reciprocal inhibition of that center by the inspiratory half-center. When the expiratory half-center takes over a reverse reciprocal interaction occurs. This agrees with the views of Brown (1911, 1914) and the alternate activity of half-centers demonstrated by Bronk and Ferguson (1935) during curari poisoning.

Now it is well known that synaptic action at the neurone membrane outlasts in varying degree the impingement of signals. If the impingement is intense and prolonged, after-discharge, which is but an indication of after synaptic action, may continue a minute or more. Such after-discharge is not regarded as abnormal by us but on the contrary as a most important phenomenon in the economics of nerve signals. Should, for example, the effects of impulses impinging at the neuro membrane be enduring, those impulses impinging on the inspiratory half-center during the expiratory phase would not be wasted for they would hold the inspiratory half-center in readiness the moment the respiratory shift occurred. In other words, they would prime the center for instantaneous activity. The same would hold for impulses impinging upon the expiratory half-center during the inspiratory phase. Thus if two nerves were simultaneously and continuously stimulated, one predominantly inspiratory and the other predominantly expiratory, a continuous source of nervous power would be available for producing a rhythmic activity through the intermediation of the precedence of stimulation inherent in the interaction of the opposing half-centers. This actually occurs when the vagus and the chemoceptors are simultaneously excited (Gesell, Steffensen and Brookhart, 1937).

The modus operandi of the principle of precedence of stimulation is more strikingly demonstrated by intermittent stimulation of a single nerve with dual excitatory action such as the cervical vagus. Should the periods

of stimulation be short and the intervening recoveries long, synaptic action of the rhythmically impinging impulses should subside between each pair of stimulations and the tendency toward sustained after-action should be largely eliminated. Afferent impulses arriving at the respiratory center during the expiratory phase should therefore reinforce the expiratory discharge with little or no effect upon the inspiratory discharge. In figure 5, for example, to the right of *B* there is a series of breaths in which each expiration is brought into phase with stimulation. Each stimulation will be seen to fall at the very beginning of expiration, a particularly effective moment for reinforcing expiration, because the inspiratory center is temporarily exhausted and the expiratory half-center is fully recovered and probably in its most responsive condition Now when the frequency of the artificial blocks of stimuli approach the natural rhythm of breathing they are very likely to set up a rhythm of their own but for reasons not yet clearly understood the capacity for establishing a new rhythm varies. In figure 5 that capacity was not pronounced for between *A* and *B* the greatest irregularity of response occurred, due no doubt to the changing incidence of stimulation. Note particularly the exceedingly intensified inspiration at *A* and *B* where stimulation falls at a moment favorable for such reinforcement.

In figure 6, on the other hand, where the frequency of breathing is low breathing was brought into phase with intermittent stimulation of twice the respiratory rhythm. Here inspiration instead of expiration falls into phase with stimulation. This more unusual phase relationship may also be tied up with the low frequency of breathing for the long expiratory pause following each artificial stimulation is conducive to recovery of the inspiratory half-center and a high susceptibility of that center to the periodically impinging signals. Conditions such as these should be favorable for a repeated selective activation of the inspiratory half-center alone. Once such a rhythm is established activation of the expiratory half-center is excluded by the subsidence of synaptic activation at the expiratory half-center during the long period of inspiratory activity remaining at the close of each artificial stimulation.

Hering's nerve. This nerve is now recognized as playing a most important rôle in respiration. Though its function differs decidedly from the pulmonary vagus we shall see that fundamentally it operates on similarly deeply rooted neurophysiological principles. Powerful faradic stimulation, as seen in figure 7, produces a marked increase in pulmonary ventilation, no doubt due to the predominance of the chemoceptor afferent signals. The deepening of respiration produced by such stimulation is a resultant of two effects, an increased strength of inspiratory contraction plus an increased strength of expiratory contraction. Both inspiration and expiration increase in depth as stimulation continues, indicating that a sum-

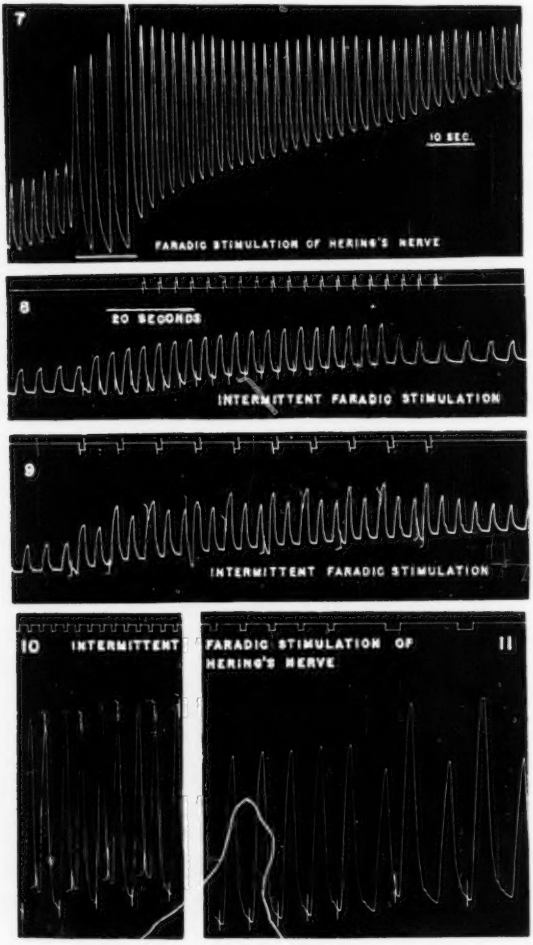


Fig. 7. Respiratory response during and after faradic stimulation of Hering's nerve showing a predominantly inspiratory action and an after discharge in both inspiratory and expiratory half-centers.

Fig. 8. Intermittently interrupted faradic stimulation of Hering's nerve in which a slowly changing time relation develops between breathing and stimulation despite a uniform frequency of stimulation. During the first eight groups of stimuli, favorable to reinforcement of expiratory contractions, the expiratory tracings fall to a subnormal level. In the later groups, favorable to stimulation of the inspiratory half-center, only the inspiratory contractions are increased.

Fig. 9. Irregular incidence of stimulation due to lack of conformation of respiratory rhythm to a lowered frequency of stimulation. Individual augmentation of

mation of stimulation occurs at both the inspiratory and expiratory half-centers. After faradic stimulation has ended, a long persisting hyperpnea of decreasing intensity follows. This indicates that the preceding stimulation was summated into a long enduring after-discharge. In contrast with the predominantly expiratory excitatory effects of vagal stimulation, the inspiratory excitatory effects are predominant for Hering's nerve. Since the after-respiratory response is virtually the same as that occurring during faradic stimulation it is believed that two powerful sustaining drives persist and that the reciprocating interaction of centers gives precedence to only one drive at a time with each turning of the respiratory phase. Combined with the earlier findings of Gesell and White (1938) of selective and adequate stimulation of the inspiratory and expiratory half-centers by careful timing of intracarotid injections of cyanide, these results indicate the existence of dual connections of each chemoceptor, one set of connections with the inspiratory half-center and the other set with the expiratory half-center.

Granting such dual central connections, comparable to those proposed for the vagal stretch receptors, it should be as easy to demonstrate a selective inspiratory or expiratory excitation with intermittent stimulation of Hering's nerve as it was for the vagus. As a matter of fact Hering's nerve seems to have a greater capacity of establishing new artificial rhythms with intermittent stimulation (see figs. 8, 9, 10 and 11). Figure 8 is a rather lucky record in that the time relation of stimulation to breathing is shifting with stimulation drawing nearer and nearer to the inspiratory phase. In the first eight breaths where the stimuli fall relatively early in the phase of expiration there is a definite expiratory as well as inspiratory response. In the remaining breaths there is only an inspiratory effect because the stimuli come too late to strengthen expiration and whatever after-action occurs at the expiratory half-center disappears by the time the expiratory phase comes round again. But why should there be a dual

inspiratory and expiratory contractions will be seen to be related to the variation of the timing of stimulation.

Fig. 10. Intermittent stimulation of Hering's nerve in which the stimuli of the odd numbered alternating group fall into the inspiratory excitatory phase of breathing and the stimuli of the even numbered alternating group fall into the expiratory excitatory phase. The first set of stimuli is seen to reinforce the inspiratory act while the second reinforces the expiratory act. Beware of the deceptive effects of the marks indicating the beginning of stimulation for they appear to extend the respiratory strokes and thus conceal the alternation of respiration.

Fig. 11. A slower intermittent stimulation of Hering's nerve than that of figure 10. In the first half of the record each stimulation hastens the appearance of inspiration and intensifies its strength. Note the late appearance and lower amplitude of inspiration 6 where artificial stimulation is missing. With the second reduction of frequency of stimulation a new paired rhythm is established in which every alternate inspiration is introduced by an artificial stimulation.

excitatory action in the first eight breaths? The answer is found by referring back to figure 7 and noting the greater intensity and duration of the inspiratory after-discharge as compared with that of expiratory half-center. It may, therefore, be safely concluded that each of the first eight blocks of stimuli, by virtue of this favorable coincidence, stimulates the expiratory half-center immediately and by virtue of the prolonged after-synaptic action of the impulses impinging at the inspiratory half-center a later stimulation of the inspiratory half-center is produced. But as soon as the artificial stimuli have shifted to a more strictly inspiratory excitatory position the expiratory action is lost.

When frequency of intermittent stimulation was reduced decidedly below the normal respiratory rhythm, it failed to impress its artificial rhythmic stimulation (see fig. 9). The result was a changing coincidence of stimulation with the respiratory act and a changing intensity of respiratory contractions comparable to the changing respirations noted for intermittent vagal stimulation in figure 5. Stimulations, occurring early in the phase of inspiration or late enough in expiration for their effects to carry over into the inspiratory phase, produced excessively deep inspirations; whereas stimulations occurring early enough in the expiratory phase to exert an expiratory action, intensified the expiratory contractions.

Thus it is necessary to conclude that a normally continuous stream of impulses flowing from the chemoceptors could, if broken into periodic blocks, act like normally recurring periodic proprioceptive impulses to produce a selective strengthening of either act of breathing. This is more strikingly illustrated in figure 10 where breathing fell into perfect rhythm with stimulation of Hering's nerve. One set of stimuli coinciding with the end of expiration or beginning of inspiration reinforced the inspiratory act, whereas the alternating sets coinciding with the beginning of expiration reinforced the expiratory act.

In figure 11 the frequency of stimulation was markedly reduced. Only inspirations were now boosted, for conditions were such that each stimulation fell late in the expiratory phase or inspiratory excitatory period. The stimuli falling in the expiratory excitatory period were missing. Each inspiration was undoubtedly strengthened by the rhythmic stimulation for when the artificial stimulus momentarily failed, as it did at inspiration 6, that inspiration was of weaker intensity. Since inspiration 6 is delayed in onset, as well as diminished in strength, stimulations 1 to 5 not only strengthened but initiated their corresponding inspirations as well. A remarkable capacity of the center to conform to stimulation is illustrated in the right half of figure 11. Although stimulation is again approximately halved, a new related rhythm is established. Each stimulus now falls into the alternate inspiratory excitatory phase and, therefore, only every other inspiration is increased in size. An alternating frequency as well as an

alternating amplitude of breathing develops because those inspirations in which stimulation is wanting are regularly delayed. The great height of the alternating inspirations, as compared with the preceding series of inspirations in the left half of figure 11 in which the same strength of stimulation was used, is most probably due to the prolongation of each individual block of stimuli and the resulting increased temporal summation. This seems a pertinent observation not only for continuing drives, which must set up a sustained summation, but also for the proprioceptive drives which last only for the duration of each phase of respiration. Granting that pulmonary inflation and increased stretching of the Golgi endings drive the inspiratory act these drives should, therefore, increase not entirely as a result of increasing stretch and increasing activity of the respective endings but as a result of temporal summation as well.

Cutaneo-sensory nerves. The cutaneo-sensory nerves carry afferent impulses from several types of receptors. Consequently artificial stimulation with the faradic current must create a heterogeneous stream of afferent impulses rather than a functionally arranged group. Sherrington (1906) attributes the main effects of such stimulation on spinal reflexes to the impulses of pain. The predominance of pain fibers in these nerves described by Ranson (see Fulton, 1938) tallies with this point of view. The universal distribution of pain endings throughout the body is well suited to provide a respiratory drive under emergencies where pain is likely to be inflicted in any part of the body. Direct experiments show great similarity of respiratory response to stimulation of cutaneo-sensory nerves at all levels of the body (Gesell and Moyer, 1935) indicating that pain provides a *general* respiratory drive lacking such local sign commonly described for spinal reflexes.

Our present results on cutaneo sensory nerve stimulation were obtained mainly on the saphenous nerve. Increased frequency of breathing is almost universal. Both inspiration and expiration are strengthened, as a rule, but in more relatively even proportions than is seen on stimulation of either the vagus or Hering's nerve (see fig. 13, and note also that the respiratory tracings are inverted in this particular record). If breathing is extremely rapid, as in figure 15, (tracings inverted again) it may be more shallow than normal. Yet it seems unreasonable to assume, even under these conditions, that the intensity of inspiratory contractions is not increased. Despite the pronounced expiratory position of the lungs, it is difficult to conceive of such intense hyperpnea in the absence of markedly strengthened inspiratory contractions. It may however be taken for granted that the expiratory component predominates. The other extreme of greater strengthening of the inspiratory act was only occasionally witnessed (see fig. 12).

Either inspiratory or expiratory effects may reveal themselves at the

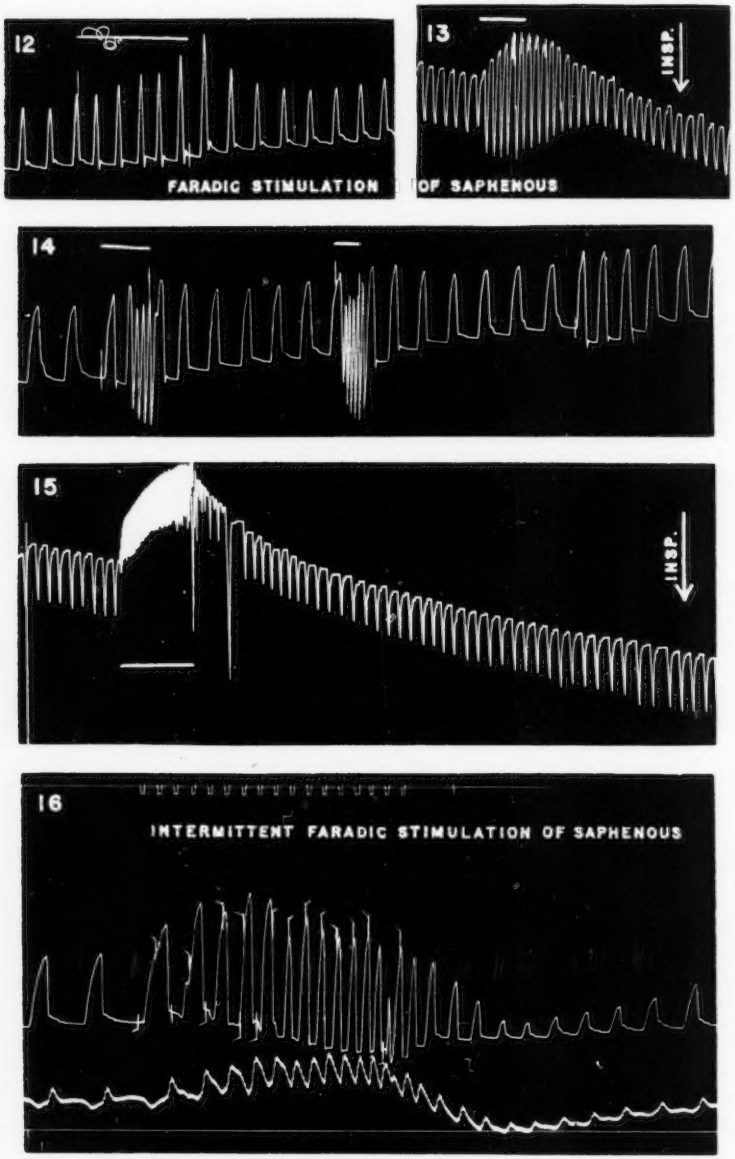


Fig. 12. Respiratory response to weak faradic stimulation of the saphenous nerve. The predominantly inspiratory action illustrated in this record is relatively uncommon.

Fig. 13. A relatively evenly balanced augmentation of inspiratory and expiratory

very onset of faradic stimulation. In the left hand record of figure 14, e.g., where the stimulation begins in the inspiratory excitatory stage of breathing, the first effect is an increased deepening of inspiration. Only as stimulation continues does the expiratory effect become apparent. In the second observation where stimulation begins in the expiratory excitatory stage increased expiratory activity is the first effect.

Figure 16 shows the respiratory response to an intermittently interrupted stimulation of the saphenous nerve. During the first five breaths of the period of stimulation in which the incidence of stimulation is changing, most of the stimulations coincide with the inspiratory excitatory phase and the main effect is a predominant strengthening of the inspiratory act, similar to results described above for the vagus and Hering's nerve. Only in respirations 3 and 5 where incidence of stimulation is more favorable to the activation of the expiratory half-center does the expiratory volume of the lungs fall below the general level. After respiration 5, a one to one correspondence of breathing to stimulation develops. Each stimulation with but one exception falls at the end of inspiration or the very beginning of expiration and expiratory activity increases progressively with each breath while inspiratory activity diminishes. The decreased breathing which ultimately occurs after the cessation of stimulation is attributable to the hypocapnic condition of the animal established by the preceding reflexogenic hyperpnea. It is concluded, therefore, that the nociceptors possess dual central connection, and that the principle of precedence of stimulation holds for the respiratory drives exerted by the nociceptor signals as it does for the chemoceptor and proprioceptor impulses.

The addition of two expiratory drives arising in diverse types of nerve fibers in conformance with the principle of precedence of stimulation. Though continuous faradic stimulation of the saphenous nerve by itself produces an

contractions produced by faradic stimulation of the saphenous nerve showing the phenomena of summation and after-discharge. In contrast to all other records, downstroke represents inspiration in this and figure 15. Vagi not cut.

Fig. 14. Faradic stimulation of the saphenous nerve beginning during the inspiratory excitatory stage in the first observation and in the expiratory excitatory stage during the second observation. The initial effects are primarily inspiratory in the first stimulation and primarily expiratory in the second.

Fig. 15. Faradic stimulation of the saphenous nerve showing powerful augmentation of respiration in which the expiratory component predominates. Vagi not cut. In contrast to all other records, downstroke represents inspiration in this and figure 13.

Fig. 16. Intermittent stimulation of the saphenous nerve in which the changing incidence of stimulation shows that stimuli falling in the inspiratory excitatory stage exert a relatively strong reinforcement of the inspiratory act whereas those falling in the expiratory excitatory stage exert a relatively strong reinforcement of the expiratory act.

increased frequency and depth of breathing, such as illustrated in figure 13, when added to a purely expiratory response of vagal stimulation it elicits surprisingly different effects. Instead of superimposing its usual expected acceleration and deepening of breathing it may only strengthen the prevailing vagal expiratory activity. This was the case in figure 17. Since both vagus and saphenous nerves carry inspiratory as well as expiratory components it may be concluded that a *selective* addition of their expiratory drives has occurred. This addition is comparable to that described in figures 5, 6, 8, 9 and 10 where the effects of short periods of inspiratory and expiratory stimulations are added respectively to the normally recurring inspiratory and expiratory activity of the respiratory center. The dis-

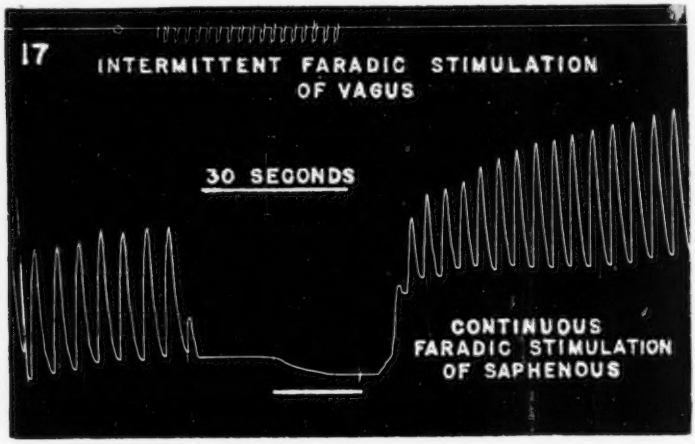


Fig. 17. Intermittent faradic stimulation of the vagus nerve producing a sustained expiratory activity and a reciprocally repressed inspiratory activity. Addition of a continuous faradic stimulation of the saphenous nerve produces a selective intensification of the vagal reflexogenic expiratory contraction.

tinguishing difference lies in the absence of normal rhythmic respiratory activity and the highly artificial conditions of the observation. *A sustained reflexogenic tetanic response to an artificial vagal stimulation has been reinforced by a sustained artificial stimulation of a second mixed nerve.* This effectiveness of the precedence of stimulation under such extremely artificial conditions in which normal activities are entirely wanting seems a most significant phenomenon for it allows a simple interpretation of a coördinated use of a heterogeneous mass of impinging signals during normal respiratory activity.

It is, therefore, of interest to determine whether similar combinations of expiratory components would yield the same results when the superior laryngeal nerve is substituted for the vagus. Figure 18 is the answer.

Addition of faradic stimulation of the saphenous nerve during a sustained reflexogenic expiratory contraction markedly increases the strength of that contraction without any outward signs of inspiratory response. Only as

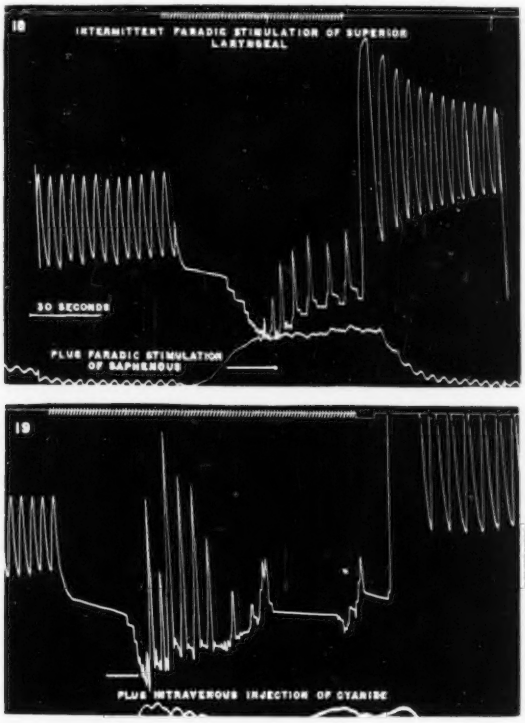


Fig. 18. Intensification of reflexogenic expiratory contraction originating in intermittent stimulation of the superior laryngeal nerve by faradic stimulation of the saphenous nerve. Continuance of saphenous stimulation leads to a temporal summation of its inspiratory component at the inspiratory half-center as is shown by the ultimate appearance and strengthening of the inspiratory act. Cessation of the predominantly expiratory stimulation of the superior laryngeal nerve in turn allows the summated action at inspiratory half-center to reveal itself in a pronounced inspiratory shift of the respiratory record.

Fig. 19. Intensification of reflexogenic expiratory contraction originating in intermittent stimulation of the superior laryngeal nerve by adequate stimulation of the carotid body chemoceptors.

saphenous stimulation continues does *inspiratory action* at the inspiratory half-center reach threshold values. Figure 19 shows the addition of the expiratory component of chemoceptor activity to the expiratory action of the superior laryngeal nerve. The addition of the expiratory component

of the noci and chemoceptive fibers to the expiratory component of either the vagal or superior laryngeal fibers is pertinent because at least four different receptor activities are involved. Such universal summation of action would seem to establish the principle that expiratory drives are additive regardless of the type of receptor in which they arise.

The addition of two inspiratory drives arising in diverse types of nerve fibers in conformance with the principle of precedence of excitatory stimulation. From the preceding results it seems that two *inspiratory* components of diverse sources should be as readily summated as two diverse *expiratory* components. Figure 20 is the answer to this assumption. The preliminary intermittent stimulation of the vagus nerve elicits a smooth expiratory contraction as in figures 17, 18 and 19. Since each vagal stimulation may be assumed to dispatch impulses to both inspiratory and expiratory half-

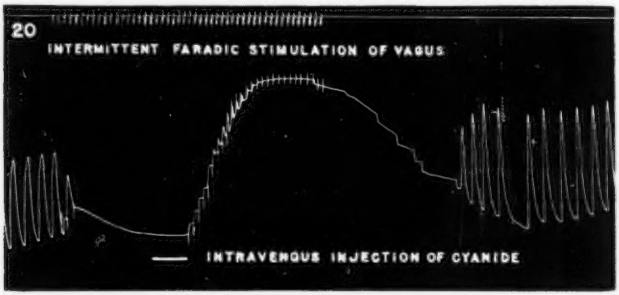


Fig. 20. Intermittent faradic stimulation of the vagus nerve and its initial expiratory action changed into a prolonged inspiratory contraction by the intravenous injection of cyanide. Selective addition of inspiratory drives is indicated by the periodic boosting of the chemoceptive reflexogenic inspiratory activity by the individual vagal stimulations.

centers, we may look upon the increasing expiratory activity as a selective temporal summation of the expiratory component of vagal stimulation. But when this expiratory contraction is changed into a prolonged inspiratory contraction by the intravenous injection of sodium cyanide the expiratory action of the vagi disappears. In its stead the inspiratory excitation comes to the fore, and with each block of intermittent stimulation the vagus reinforces the slowly rising inspiratory contraction elicited by the chemoceptive stimulation of the cyanide. In other words, a selective addition of two inspiratory effects of two groups of impulses possessing a potential power of dual excitatory stimulation has occurred—one group of impulses coming from the chemoceptor and the other from the vagus.

Apparent exceptions to the principle of precedence of stimulation. With the aid of the principle of precedence of stimulation it is now possible to exercise a highly predictable experimental control of the respiratory act

and for that reason we are inclined to believe that this principle represents a key phenomenon in nervous integration not only for the respiratory act but for other motor integrations as well. Why then are there deviations from the rule such as the checking of the inspiratory act by artificial vagal stimulation occurring during the inspiratory phase of breathing (see fig. 17)? This question is analysed with the aid of the simplified schema (fig. 21) resolving our working hypothesis to its very simplest terms (for a more detailed account, see Gesell, 1940e). The possible rôles of the internuncial neurones are, therefore, not included. Suffice to say that normal nerve cell activity is regarded as a *rhythmical phenomenon* resulting from the action of an electrotonic current emerging at the axon hillock. This current, generated by a metabolic gradient, is graded by the sum total of impinging signals. Each receptor, of the types illustrated, is assumed to impress a double drive, one upon the inspiratory half-center and the other upon the expiratory half-center. These centers are figuratively represented by single cells. Division of each afferent fiber coming from the individual receptors accomplishes the double drive, one branch synapsing at the inspiratory half-center and the other at the expiratory half-center. The boutons of these two divisions are allotted in round proportions (not numbers, for hundreds or even thousands may impinge upon a single cell) roughly in relation to experimentally observed activity described above. For the predominantly expiratory stretch receptor, four expiratory and two inspiratory boutons are allotted; for the predominantly inspiratory chemoceptor, four inspiratory and two expiratory boutons; and for the more evenly balanced pain receptor, four inspiratory and four expiratory.

Each discharging receptor impresses its effects simultaneously on both the inspiratory and expiratory cells, but thanks to the reciprocating connections a coördinated alternating activation is attained. Suffice to say that the negativities at the reciprocating boutons, strategically and hypothetically placed to oppose the outflow of the *excitatory* electrotonic currents at the axon hillocks, are pictured as diminishing and therefore grading the activity of the opposing half-center (Gesell, 1940e).[3] If it be granted that the reciprocal inhibition of the expiratory half-center by the inspiratory half-center during the inspiratory phase of breathing is sufficient to keep the electrotonic excitatory currents of the expiratory half-center below threshold values of the individual axon hillocks, the inspiratory center

[3] Obviously other mechanisms of inhibition might be proposed such as the deposition of oppositely acting chemicals at excitatory and inhibitory synapses. One action might be an increased permeability of the underlying membrane increasing the local negativity, the other action might be a decreased permeability decreasing the local negativity. The view employed in our present paper must be regarded only as a working hypothesis.

alone becomes open to reflexogenic reinforcement. Under these conditions theory demands that one inspiratory excitatory component be added to the other. Conversely if the inspiratory half-center is adequately inhibited by the activity of the expiratory half-center expiration only is open to reflexogenic reinforcement. In this situation one expiratory excitatory component is added to the other to the exclusion of inspiratory activity.

How then, specifically, can vagal stimulation during the inspiratory phase check the inspiratory act as it did in figure 17 if reflexogenic inspiratory inhibition (i.e., direct and not reciprocal inhibition) be dismissed? Total absence of an inspiratory excitatory component would be an adequate and also the simplest answer, because the only other conceivable effect would then come from an activation of the expiratory half-center. If it then be granted that the impulses impinging on the expiratory half-center be sufficient to overcome the reciprocal inhibition exerted by the normal rhythmic discharge of the inspiratory half-center, the expiratory half-center would in turn discharge and thereby reciprocally inhibit the inspiratory half-center. Another possible explanation of figure 17 is that the normal inspiratory discharge was insufficiently established to insure a *highly protective* reciprocal inhibition of the expiratory center. In that event the expiratory half-center would be more susceptible than usual to excitation, particularly if the expiratory component of the stimulation were strongly developed, for it seems fair to assume that an extremely powerful expiratory stimulation would be capable of overcoming the reciprocal inhibition exercised by the normal discharge of the inspiratory half-center plus a weak inspiratory reflexogenic reinforcement. On this basis conditions are conceivable in which artificial stimulation of a mixed nerve may either reinforce or suppress the inspiratory act during the inspiratory phase of breathing.

An interpretation of the combined inspiratory and expiratory drives of normal breathing. When expiration is purely passive the respiratory act theoretically becomes a relatively simple nervous integration, for breathing then may resolve itself primarily into an inspiratory phenomenon uncomplicated by the interaction between half-centers. Accordingly each inspiratory act becomes a purely self limited activity, in which the inspiratory discharge comes to a natural end in consequence of a normal exhaustion of the inspiratory cells (Gesell, Atkinson and Brown, 1940). According to this conception the inspiratory cells discharge at regular intervals with a rhythm determined by the rate of recovery of the threshold excitability after each preceding discharge. But when expiratory activity is deliberately introduced by stimulating the superior laryngeal or vagus nerve, as was done in figure 22, the machinery of breathing is importantly changed. Impulses now cross in the diagram from the discharging expiratory cells,

via the reciprocating collaterals, to the inspiratory cells and thus reduce the electrotonic excitation current of these cells below threshold value. The inspiratory cells consequently cannot discharge unless stimulation (i.e., the hypothetical electrotonic current) builds to *higher* values and counteracts the E.M.F. imposed by reciprocal inhibition. Due to the increasing

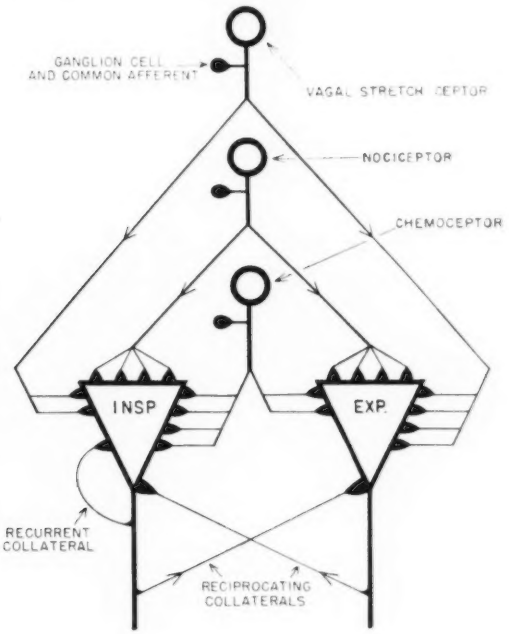


Fig. 21. A schema of the electrotonic theory of nervous integration and of the principles of reflexogenic drives reduced to simplest conceivable outline. The inspiratory and expiratory cells represent their respective centers. These centers are pictured as being driven by three representative sets of impulses arising in three groups of receptors important to the respiratory act. Each individual receptor communicates its effects through the final terminating boutons of the individual divisions of the original afferent fiber. The relative strengths of the inspiratory and expiratory components of each group of impulses are indicated roughly by the number of impinging synapses. Rhythmical alternating activity is attained by the interaction of the reciprocating collaterals. For simplicity internuncial neurones are not included in present considerations.

carbon dioxide and the decreasing oxygen, both centrogenic and reflexogenic drive increase and cancel the deficit of electrotonic current established by reciprocal inhibition. As the intensity of the chemical drive continues to increase following the period of almost total apnea the depth of breathing grows in corresponding proportions.

The inspirations occurring during faradic stimulation of the saphenous are markedly increased which suggests that the opposing action of the superior laryngeal nerve is overcome by noci—as well as by chemoceptive signals.

In figure 23 the superior laryngeal nerve is activated again with an intermittently interrupted faradic stimulation and inspiration is held in abeyance, as it was in figure 22. Cyanide is now injected during this period of inspiratory inactivity and rhythmical breathing comes on at once. Since it is generally agreed that cyanide stimulates breathing in the anesthetized animal mainly through its action at the chemoceptors and since inspirations disappear again as the temporary action of cyanide subsides, it may be concluded that the adequate stimulation of the chemoceptors builds up the stimulation at the inspiratory half-center sufficiently to over-

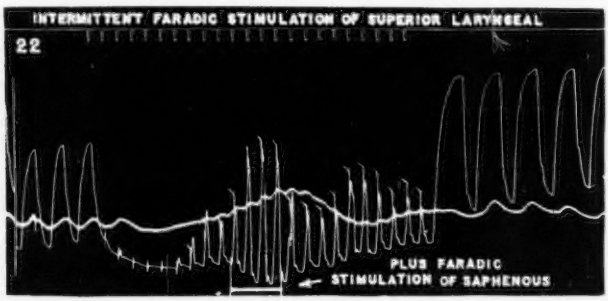


Fig. 22. A predominantly expiratory activity produced by intermittent stimulation of the superior laryngeal nerve is converted into a progressively increasing pulmonary ventilation superimposed on a newly established expiratory level by a normally increasing asphyxial chemical drive. The inspiratory and expiratory drives of saphenous stimulation add their effects to the newly created conditions established by stimulation of the superior laryngeal nerve.

come the augmented reciprocal inhibition coming from the expiratory half-center. Now that inspirations are reinstated the rhythmic stimulation of the superior laryngeal nerve plays a new rôle. While the effects of the superior laryngeal nerve are no longer powerful enough to maintain breathing in a sustained expiratory contraction, they are still effective in initiating expiratory activity at the close of every inspiratory act; for if the record be examined carefully stimulation will be found to coincide with the ends of inspiration. The ratio of breathing is exactly 1 to 4 (in one instance 1 to 5) stimulations of the superior laryngeal. Still closer inspection of the record shows that the intervening stimuli between the long expiratory excursions produce step-like augmentation of the main expiratory act.

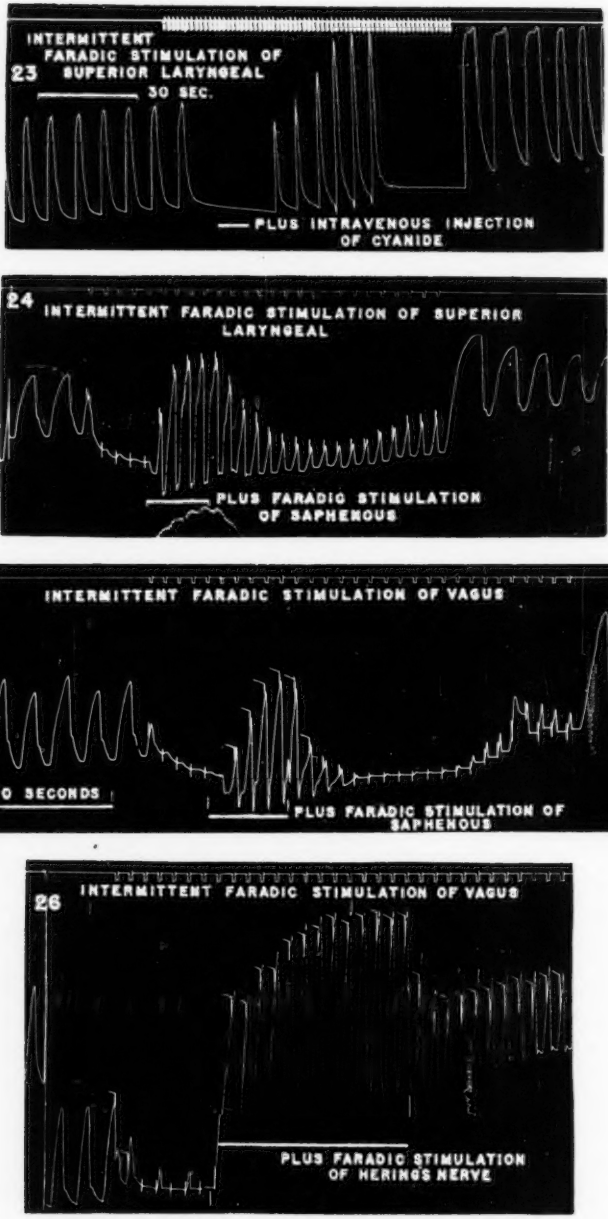
In figure 24 stimulation of the saphenous nerve is substituted for chemo-

ceptive stimulation during a period of increasing expiratory activity produced by intermittent stimulation of the superior laryngeal nerve. The first effect is a slight accentuation of the existing expiratory contraction, but that effect gives way immediately to a deep inspiratory contraction which at its crest is transformed into a sharp expiratory contraction by the next superior laryngeal stimulation. One deep inspiration follows another as a result of nociceptive reflexogenic stimulation of the inspiratory half-center and, as the center repeatedly exhausts itself with each inspiratory discharge, it becomes susceptible to the combined interrupting influences of the expiratory components of the saphenous plus the superior laryngeal stimulation. Careful inspection will show that expiration occurs in each instance immediately after the incidence of the intermittent stimulations of the superior laryngeal nerve. The increasing amplitude of inspiration during the period of stimulation of the saphenous nerve indicates a temporal summation of the inspiratory drive from the saphenous nerve in the inspiratory half-center and the decreasing amplitude after the end of stimulation indicates the dwindling of the after inspiratory action.

The results in figure 25 where a continuous faradic stimulation of the saphenous nerve are added to a sustained expiratory action of vagal reflexogenic origin, are in principle the same as those in figure 24. Both inspiratory and expiratory contractions are augmented by stimulation of the saphenous nerve as they are in figure 13. As summation of the inspiratory component of the saphenous stimulation progresses in the inspiratory half-center the depth of inspiration increases and each inspiratory act gives way to expiratory activity immediately after the beginning of each vagal stimulation. Both inspiratory and expiratory after-discharge vanish quickly after the end of saphenous stimulation. The expiratory action of the vagus gains predominance and only the faintest inspiratory contractions occur with each stimulation. These small inspiratory efforts represent the respiratory response to the relatively weak inspiratory component impressed along with the predominant expiratory component. This inspiratory effect, however, gains in strength as the inspiratory stimulation of growing asphyxia increases. Thus one inspiratory drive seems to add to a second inspiratory drive in a most effective way.

In figure 26 continuous faradic stimulation of Hering's nerve is added to an intermittently interrupted stimulation of the vagus nerve. The stimulation of Hering's nerve creates a powerful inspiratory drive and each inspiration so produced is followed by an expiration initiated at regular intervals by the intermittent stimulation of the vagus.

It may, therefore, be concluded (see figs. 22, 23, 24, 25 and 26) that an increased activity of the expiratory half-center artificially produced by faradic stimulation of predominantly expiratory nerves, such as the vagus or superior laryngeal, exerts a suppressing action upon the inspiratory


23
INTERMITTENT FARADIC STIMULATION OF SUPERIOR LARYNGEAL
30 SEC.
PLUS INTRAVENOUS INJECTION OF CYANIDE
24
INTERMITTENT FARADIC STIMULATION OF SUPERIOR LARYNGEAL
PLUS FARADIC STIMULATION OF SAPHENOUS
25
INTERMITTENT FARADIC STIMULATION OF VAGUS
20 SECONDS
PLUS FARADIC STIMULATION OF SAPHENOUS
26
INTERMITTENT FARADIC STIMULATION OF VAGUS
PLUS FARADIC STIMULATION OF HERINGS NERVE

Figs. 23–26

half-center. This effect, however, may be overcome by intensified inspiratory drives (centrogenic chemical, reflexogenic chemical and reflexogenic pain) which reëstablish rhythmic activity in the inspiratory half-center. The residual vagal proprioceptive stretch reflex obtaining under physiological conditions during the expiratory phase of breathing (Adrian, 1933) is theoretically capable of initiating expiration at the end of inspiration and of restraining inspiration during the expiratory phase in a manner comparable to that of artificial rhythmic stimulation seen in figures 22, 23, 24, 25 and 26. But the simultaneous impingement of vagal impulses at the inspiratory and expiratory half-centers during normal activity of the respiratory center call for a more detailed discussion of the mechanism of the normal changing of the inspiratory into the expiratory phase of breathing.

The turning point of inspiration into expiration. Assuming that the discharge of the inspiratory half-center is a self limited activity coming to an end *solely* as a result of "functional exhaustion," intensification of that discharge might therefore intensify the functional exhaustion and thereby bring the discharge to a premature end. Since evidence is now at hand that pulmonary inflation actually does *intensify* and *shorten* the inspiratory discharge as evidenced by action potential studies (Worzniak and Gesell, 1939) the rôle of a simultaneously intensified exhaustion of the inspiratory half-center calls for consideration. The difficulty of a clean cut analysis of the factors involved comes from the simultaneity of impingement of vagal impulses upon the inspiratory and expiratory half-centers and the interaction of these half-centers. There is a probability that the inspiratory exhaustion and the reciprocal inhibition may be working in such in-

Fig. 23. A sustained expiratory contraction produced by intermittent stimulation of the superior laryngeal nerve is interrupted by intravenous injection of cyanide. Breathing conforms with superior laryngeal stimulation in ratios of 1 to 4 (once in the ratio of 1 to 5) in which each inspiration is interrupted by a superior laryngeal stimulation. After the subsidence of the predominantly inspiratory action of cyanide, the superior laryngeal stimulation regains complete control of breathing.

Fig. 24. A predominantly expiratory response produced by intermittent stimulation of the superior laryngeal nerve is changed into rhythmical breathing by faradic stimulation of the saphenous nerve. During this artificial hyperpnea superior laryngeal stimulations occur at the height of inspirations thus preceding the following expirations.

Fig. 25. A predominantly expiratory response produced by intermittent stimulation of the vagus nerve is converted into a hyperpnea with expiration in phase with vagal stimulation by continuous faradic stimulation of the saphenous nerve. Note that summation and after-discharge from saphenous stimulation are as marked on a background of exaggerated as on normal expiratory activity.

Fig. 26. A predominantly expiratory activity of reflexogenic vagal origin is converted into hyperpnea in phase with vagal stimulation by faradic stimulation of Hering's nerve.

timate correlation as to defy differential evaluation. Sherrington and Sowton (1940), e.g., have shown that a long lasting spinal reflex is more readily inhibited than the same reflex of shorter duration and conclude that fatigue favors inhibition. Our figure 27 shows the same phenomenon in prolonged inspiratory contractions subjected to a series of combined stimulations of the superior laryngeal and saphenous nerves in which the expiratory component may be regarded as predominant. It will be seen that the reciprocally inhibitory stimuli become more effective the later their occurrence in the inspiratory act. Since an increasing tempo of the individual inspiratory activity patterns of the inspiratory half-center favors the development of functional fatigue just as does a prolongation of a single inspiratory activity the intensification of the inspiratory act produced by the normal vagal stretch reflex would also increase its susceptibility to inhibition. This ties the two inspiratory interrupting forces of

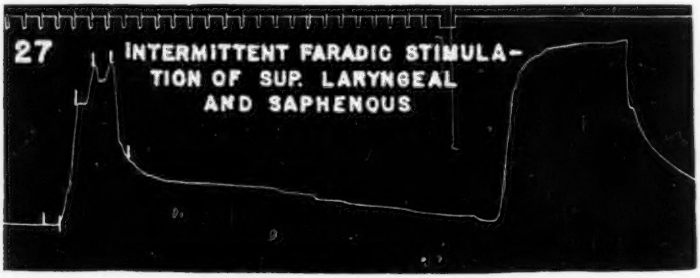


Fig. 27. The effects of a mixed stimulation of the superior laryngeal and saphenous nerve upon a slowly developing inspiratory act. The effectiveness of the combined expiratory drives of these two nerves in checking inspiration is greater the later the stimulation falls in the inspiratory act.

the vagal stretch reflex into a coördinated dovetailed arrangement. Pulmonary stretch tends of itself to shorten inspiration through exhaustion but by this very exhaustion it also prepares the cells for a premature reciprocal inhibition produced by the simultaneous reflexogenic excitation of the expiratory half-center.

Paradoxical acceleration of breathing. Some years ago effects of combined stimulation of the vagus nerve and the chemoceptors were described by Gesell, Steffensen and Brookhart (1937). As is well agreed a strong stimulation of the vagus nerve produces a very slow frequency of breathing with long expiratory phases. Similarly a powerful stimulation of the chemoceptors with cyanide during double vagal block may produce slow breathing with prolongation of the inspiratory contractions. When however these two effects are combined there is not a combined slowing of respiratory rhythm, but rather an extraordinary acceleration. At the

time, these results seemed very strange to the authors which led them to use the term of "paradoxical acceleration"; but in the light of present findings, this acceleration seems to have a simple and logical explanation. This hinges on the schematic representation of the vagal and the chemoceptive drives in figure 21. When vagal and chemoceptive stimulations are combined, both inspiratory and expiratory drives add up to six (2 + 4 and 4 + 2). This may be interpreted to imply, in a rough way to be sure, that the inspiratory and expiratory drives are evenly balanced, that neither center is in position to become predominantly active, and that conditions are such as to bring about a rapid alternation of activity. That tallies with our evidence for a relatively evenly balanced inspiratory and expiratory drive of the nociceptors and thus becomes a theoretical explanation of the well known acceleratory effects of stimulation of cutaneo-sensory nerves.

SUMMARY AND CONCLUSIONS

Experiments were devised to analyze the machinery of the reflexogenic drive of the respiratory act. Three types of nerves were stimulated and their effects observed upon the frequency and depth of breathing. The vagus nerve was chosen for the proprioceptive fibers it contains, Hering's nerve for the high content of chemoceptive fibers and the saphenous for the predominance of nociceptive fibers.

Faradic stimulation of the vagus nerve which began during the expiratory phase intensified and prolonged the period of expiratory activity thus preventing the normally recurring inspiratory cycles. When stimulation began in the inspiratory phase the inspiratory act was frequently intensified. This inspiratory response gave way immediately to a sustained expiratory response similar to that produced when stimulation begins in the expiratory phase. Because the expiratory effects are more prolonged and seemed to be more powerful the vagus was regarded as impressing a predominantly expiratory drive.

Faradic stimulation of Hering's nerve was found to produce a rhythmic form of breathing, slower or faster than normal, in which the depth of inspiration and expiration were both increased. The much greater inspiratory action classifies this nerve as predominantly inspiratory.

Faradic stimulation of the saphenous nerve was found to produce a rapid, rhythmic form of breathing in which the intensity of both inspiration and expiration were often equally increased. The relatively more even balance of inspiratory and expiratory action places the effects of cutaneo-sensory nerves approximately midway between the vagus and Hering's nerve.

Intermittently interrupted faradic stimulation of any of these dual excitatory nerves devised to vary the incidence of stimulation with respect

to the phase of the respiratory act elicited a selective excitation of either the inspiratory or expiratory half-center depending upon the phase of activity of the respiratory center existing at the moment of stimulation.

Since stimulation of any mixed nerve reinforcing both acts of breathing assumably increases the signals impinging on both half-centers, it is concluded that susceptibility of each half-center to stimulation depends upon the phase of activity then prevailing. This tendency of selective activation of normally discharging half-centers is designated as the principle of precedence of stimulation.

This principle is demonstrated to hold for more abnormal situations as well. If rhythmic respiratory activity is abolished and replaced by a prolonged artificial expiratory contraction (by stimulation of either the vagus or superior laryngeal nerve) that contraction is intensified without inspiratory complications by stimulation of the saphenous or Hering's nerve. This illustrates a selective addition under highly artificial conditions, of diverse expiratory components out of two highly differing nerves.

When a sustained expiratory activity produced by intermittently interrupted faradic stimulation of the vagus nerve was changed into a slowly developing inspiration by the intravenous injection of sodium cyanide, each vagal stimulation then reinforced the chemoreflexogenic inspiration. Thus diverse inspiratory components of decidedly different afferent nerves were selectively added by bringing inspiratory activity to the fore.

The demonstrable summation of reflexogenic drives arising in diverse types of receptors indicates a common action of their impinging signals at the receiving neuromembrane and the primary importance of the principle of the precedence of stimulation.

In confirmation of earlier considerations the *sum total of impinging signals constitutes the power which drives the central nervous system.* Just as a sustained predominantly expiratory drive may hold respiration in the expiratory phase so may a predominantly inspiratory drive tend to hold respiration in the inspiratory phase. Combine these temporally unrelated mass drives and a rapid alternating respiration is the result.

The relatively even balance of inspiratory and expiratory components in cutaneo-sensory nerves is thought to explain their highly acceleratory action on the frequency of breathing.

The principles here summarized were used to present a hypothetical picture of the neuromachinery of normal breathing.

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THE INFLUENCE OF THE CERVICAL SYMPATHETIC NERVE ON THE LENS OF THE EYE

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In a series of papers (Morgan and Olmsted, 1939; Olmsted and Morgan, 1939; Morgan, Olmsted and Watrous, 1940) we have shown that the sympathetic nervous system may play a definite rôle in accommodation of the mammalian eye for far vision. The method we used for measuring changes in the dioptric power of the lens in the cat, dog and rabbit was the one commonly used for the human eye, skiascopy. Although this is an "objective" method of measurement, it was thought advisable to present, if possible, photographic evidence of change in the curvature of the lens during stimulation of the cervical sympathetic nerve.

With this end in view we first attempted to photograph the three Purkinje-Sanson images reflected from the eye of the rabbit under light ether anesthesia before and during sympathetic nerve stimulation. The image reflected from the anterior surface of the lens, however, proved to be so diffuse that, although a shift in its position could be readily detected in enlarged photographs, as well as on direct observation, it was not sharp enough to permit of reproduction. Accordingly, outline tracings have been made from such photographs to show diagrammatically the extent of movement observed. It will be seen from figure 1 that one, and only one, of the three images shifts its position upon stimulation of the cervical sympathetic. The right hand image approaches the center one.

The classic picture of the three Purkinje-Sanson images portrays a change in position of the center image on accommodation to far vision. This is because the Helmholtz phacoscope, through which the images are ordinarily observed, is so arranged that the image reflected from the anterior surface of the lens lies between the image from the cornea and that from the posterior surface of the lens. In our photographic set-up the source of light was on the left of the subject's (left) eye as in Helmholtz' arrangement, but the camera, instead of occupying the position of the observer's eye at a corresponding angle on the right, was for convenience placed directly in front of the subject's eye. A drawing to scale of the eye and of the incident and reflected rays showed that when viewed from in front the image reflected from the anterior surface of the lens should be to

the right of the other two images, and that with decreased curvature of the anterior face of the lens the image from this reflecting surface should move in toward the image from the cornea. The extent of such excursion would be slight. Since the photographs show results so completely consistent with those demanded by theory, we may safely infer that stimulation of the cervical sympathetic does cause a decrease in curvature of the anterior face of the lens.

A second method, that of photographing the profile of the lens directly, yielded the desired result. We had found by skiascopy that the range of accommodation in the cat is greater than in the rabbit, 4–5 diopters as compared with 2–3, the extreme measurements being taken during stimulation of the third nerve and during stimulation of the cervical sympathetic.

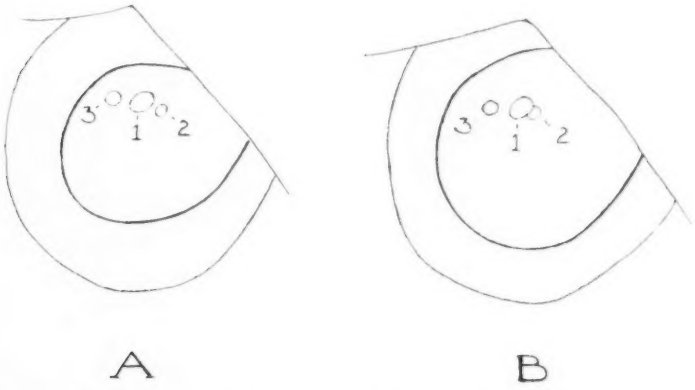

Fig. 1. Outline tracings of photographs of the three Purkinje-Sanson images reflected from a rabbit's eye; A, before stimulation of the cervical sympathetic nerve; B, during stimulation.

In one exceptional cat the total range was 12 diopters. The decrease in the dioptric power of the lens of the cat under light anesthesia upon stimulation of the cervical sympathetic was between 1 and 2 diopters. The normal cat eye, however, is not practicable for photographing changes in the lens because of the narrow slit-like iris in bright light which leaves so little of the lens exposed. Consequently we performed partial iridectomy on three cats, removing the outer and lower part of the iris of the left eye. Two months were allowed for healing of the slit in the cornea through which the iris had been removed, and for resorption of the blood clot. In these partially iridectomized cats we have succeeded in photographing the anterior surface of the lens by means of a special lighting system modeled on the one used by Fincham (1935) for photographing the lens of a human patient without iris. Because the upper eyelid interfered somewhat with

the view, the camera was not placed strictly vertically over the cat's head but about 15° off the perpendicular. Our photographs, therefore, do not show the exact equator of the lens where we should expect evidence of greatest change, but a profile slightly above the equator.

Comparison of the two photographs in figure 2 taken of the same eye of a lightly anesthetized cat before and during sympathetic stimulation shows a distinct flattening of the anterior surface of the lens during sympathetic stimulation. It will be noted that the eyeball is protruded during sympathetic stimulation, but because of the position of the camera and its distance from the eye, the two photographs still coincide when superposed in spite of this slight forward movement. Had, however, the eyeball been

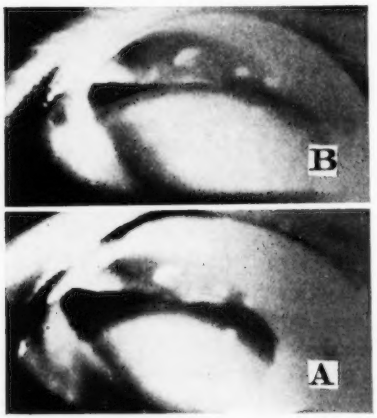

Fig. 2

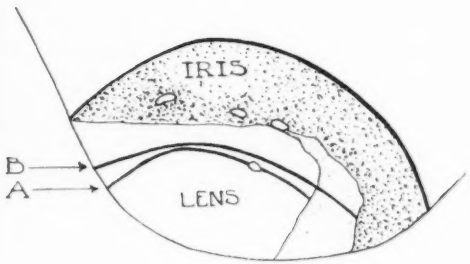

Fig. 3

Fig. 2. Photographs of the lens profile of a partially iridectomized cat; A, before stimulation of the cervical sympathetic nerve; B, during stimulation.

Fig. 3. Superposed outline tracings of the photographs shown in figure 2.

rotated during the interval between the taking of these two photographs, the interpretation would be in doubt. Because the lens is not a perfect sphere, even slight rotation would change the configuration of its profile as seen from the camera. It will be noted that the position of the series of small reflections from scars and imperfections of this cornea and the droplets of saline to keep the cornea from drying remains the same in both photographs as is shown in figure 3 where outline drawings of the two photographs are superposed.

SUMMARY

Photographs of the Purkinje-Sanson images reflected from the eye of a rabbit show that the image from the anterior face of the lens changes its

position during stimulation of the cervical sympathetic in the direction which would be demanded if such nerve action resulted in a decrease in curvature of the anterior face of the lens. Photographs of the anterior face of the lens in partially iridectomized cats under light ether anesthesia show that the anterior face of the lens does actually flatten during stimulation of the cervical sympathetic nerve.

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THE SLOW COMPONENTS OF THE ELECTROGRAM OF STRIATED MUSCLE

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Unlike the spike, the slow components of the electrogram of striated muscle have been the object of only few studies. The conducting system of striated muscle has been found to differ only quantitatively from that of nerve. The electric phenomena which attend conduction in nerve are the spike potential and the negative and positive afterpotentials. A similar sequence of electrical changes is to be expected in striated muscle. The interpretation of the electromyogram, however, is complicated by the fact that muscle is not only a conducting but also a contracting tissue. Contraction occurs at the time when the afterpotentials of conduction would be developing. It is possible that the physicochemical changes underlying contraction have an electric manifestation. In the presence of slow potential changes during contraction, therefore, it is difficult to decide whether they are associated with one or the other of the two processes, conduction or contraction, or whether the electric manifestations of both are coincident and interact.

This study deals with the analysis of the several components of the electrogram of striated muscle and of their relations to the functions of the tissue.

METHOD. Cats were used, anesthetized with dial (Ciba, 0.75 cc. per kgm., intraperitoneally). The muscle studied was usually the sartorius. In some observations it was denervated by previous (7 to 15 days) section of the femoral nerve under ether anesthesia and with aseptic precautions. The soleus muscle was observed only occasionally for comparison with sartorius. The description of the methods used will refer to the latter muscle; the obvious slight differences of procedure which took place when soleus was employed will not be outlined.

The leg was fixed by means of drills inserted into the femur. The tibial end of sartorius was tied and separated from the bone. It was then attached to a torsion spring myograph of the Sherrington type. The contractions were isometric. They were recorded simultaneously with the

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electrograms by sending the beam of reflected light from the mirror in the myograph to the back of the film in the camera.

The leads for recording the electric responses were large chlorided silver needles inserted as follows. The muscle was crushed about 0.5 cm. below the tie at the tibial end. One electrode was between the tie and the crush. The other one was in normal muscle, about 1.5 cm. below the crush. After the electrodes had been inserted the exposed part of the muscle was covered with vaseline in order to prevent drying.

A 5-stage direct-coupled amplifier was used. A battery in series with the muscle and with a high input resistance provided a counter e.m.f. which balanced the potential difference between the electrodes. This potential difference was due mainly to the demarcation potential of the muscle. Only exceptionally was a capacity-coupled amplifier employed to observe the spike potentials with little shift of the base line—i.e., with filtration of the slow components. The amplified responses were photographed from a cathode ray oscillograph.

The stimulating electrodes were shielded silver wires applied to the femoral nerve, crushed or cut centrally. Activity in the quadriceps muscle was prevented from interfering with the records by section of either the corresponding motor nerve or the patellar tendon.

The stimuli were condenser discharges through a thyratron. They were rendered diphasic by means of a transformer. Injections were made into the central end of the cannulated inferior mesenteric artery.

Results. A. *The electrogram of normal muscles.* With the method used the records were usually monophasic—i.e., there was little or no indication of a diphasic artifact at the end of the spike potential. The monophasicity of the responses was maintained for the duration of the experiments (up to 7 hrs.) without recrushing the muscle. The stability of the preparation was further demonstrated by the constancy of the demarcation potential. The value of this potential was usually about 15 mv. Unless drugs were injected it remained practically unchanged throughout the observations.

A typical response of sartorius to a single maximal motor nerve volley is illustrated in figure 1. Three slow components may be recognized following the spike: a first negative wave, then a relatively positive excursion, and finally a prolonged second negative deflection. Frequently, but not invariably, the positive wave was roughly coincident with the development of tension in the mechanogram.

Although this complex sequence of potentials was the rule, occasionally quite different electrograms were encountered (see fig. 4A). Any of the slow components mentioned could be minimal or absent. The slow potentials could consist of a single negative or positive wave. Some of the slow components, however, were always present—i.e., there was never any record exhibiting exclusively a spike potential.

The effects of repetitive stimulation of the motor nerve varied with the frequency applied (fig. 2). During the period of stimulation there was summation of some of the slow components elicited by a single volley. Thus, an increasingly negative background for the successive spikes was seen for frequencies of 10 to 30 per sec. Between 30 and 100 per sec. less negativity was usually developed than with the slower frequencies; indeed, occasionally positivity, instead of negativity, summed during the period of stimulation at these intermediate frequencies.

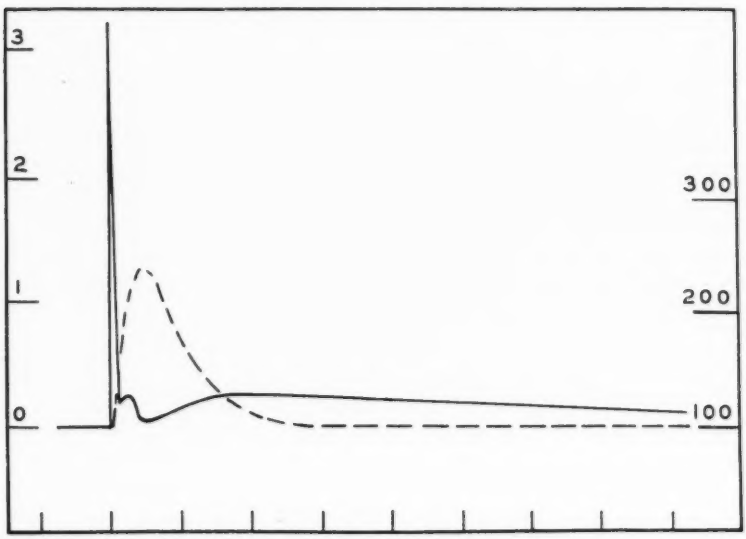

Fig. 1. Electrical (solid line) and mechanical (broken line) responses of sartorius to a single maximal nerve volley. Enlarged and superimposed from the original film. Time scale (abscissae): 100 msec. Left scale of ordinates, mv.; and right scale, tension in grams.

The effects of rapid rates of stimulation (100 to 600 per sec.) were typically as follows. The spikes promptly declined to a small fraction of the initial amplitude. The background potential was first negative, then relatively positive (that is, less negative), then again increasingly negative. With the highest frequencies mentioned (500 to 600 per sec.) the mechanogram exhibited an initial rise of tension followed by a rapid fall, and later by a slow second rise. The late slow rise of tension was accompanied by a parallel shift of the electrogram in the positive direction in 3 out of 5 animals. In the other 2 this shift was toward increased negativity.

The after-effects of a period of repetitive stimulation showed a slow (15 to 120 sec.) return toward the resting condition. With frequencies

greater than about 30 per sec. a positive swing occurred shortly after the end of stimulation (fig. 2, D and E). This excursion toward positivity was followed by increased negativity. The time course of this positive swing had no relation to the period of relaxation of the muscle; thus it could take place either toward the end of relaxation or later. Occasionally two positive waves, instead of one, could be seen at the end of a period of stimulation.

B. *The effects of veratrine.* Injections of this drug resulted in striking changes in both the mechanogram and the electrogram of the muscles

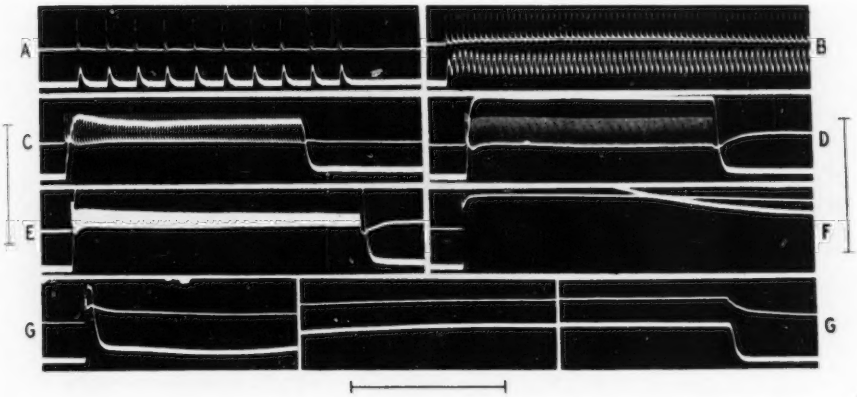

Fig. 2. Responses of muscle to repetitive indirect stimulation. Frequencies: A, 2.5; B, 13; C, 26; D, 52; E, 110; F. 250; and G, 600 per sec. The intervals in record G correspond to 2 and 4 sec., respectively; the stimuli were applied continuously.

In this and the following figures the records are from sartorius unless otherwise stated. The lower tracing is the mechanogram and the upper tracing the electrogram. Upward deflections in the electrogram denote negativity of the intact with respect to the crushed part of the muscle. The vertical lines at the left calibrate the electric responses; those at the right, the mechanical responses. The horizontal lines show the speed of the records. For this figure these calibrations are as follows: 10 mv.; 1 kgm.; and 2 sec.

studied. There was considerable variability from animal to animal in the dose of veratrine which produced a given effect. Thus, while 1 mgm. per kgm. caused in some cats a marked reduction of the responses, which disappeared only after 1 to 2 hours, in other cats 6 to 8 mgm. per kgm. could be injected over a period of 15 to 30 minutes before any significant reduction of the responses was seen. Because of this variation of susceptibility the expressions "small," "medium" and "large" doses of veratrine will be used with reference to the particular animal under consideration. A "large" dose is that which resulted in prolonged depression; a "small" dose, that

which produced only a moderate degree of repetition and a moderate increase of the residual negativity; finally, a "medium" dose is that which elicited marked repetition and great residual negativity.

The demarcation potential was consistently increased by veratrine. The change was usually slow—i.e., it took place over a period of minutes after the injection. Successive injections caused increasing effects. The largest change seen was an increase from 14 to 33 mv. after injection of 5 mgm. per kgm. of veratrine in successive doses of 1 mgm. over a period of 3 hours.

The residual negativity of the muscle after single maximal stimulation of the nerve was greatly increased by veratrine (fig. 3). The peak of this negativity was frequently higher than the peak of the initial spike poten-

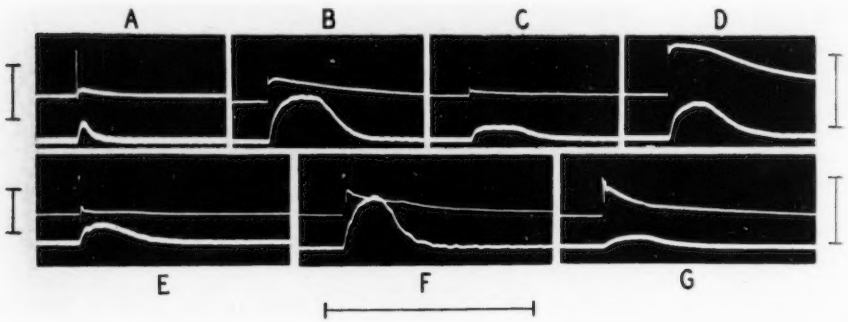

Fig. 3. Effects of various doses of veratrine on the muscular responses to indirect stimulation by single maximal shocks.

A to D, sartorius. A: normal response. B to D: 0.5, 8 and 53 min., respectively, after veratrine (1 mgm. per kgm.). Calibrations: 5 mv.; 200 grams; and 2 sec.

E to G, soleus. E: normal response. F and G: after injections of veratrine (1 and 8 mgm. per kgm., respectively). Calibrations: 10 mv.; 1 kgm.; and 2 sec.

tial. The negativity grew for some time after subsidence of the spike, and then declined slowly. The decline was not uniform or smooth. A relatively positive hump was usually apparent shortly after the peak of the negativity and occasionally two such positive humps were visible (fig. 4).

Repetitive stimulation of the nerve at slow frequencies after small doses of veratrine resulted in a marked summation of negativity. With frequencies higher than 20 per sec., however, less negativity was developed than normally. The positive swing after cessation of the stimuli was well-marked in these conditions. With moderate doses of veratrine there was more negativity than normal, both during and after the period of stimulation, for any frequency. Both the early positive excursion during

stimulation and the one following cessation of high frequencies were not detectable.

The spike responses of muscle to single shock stimulation of the nerve were repetitive after veratrine (fig. 5). This repetition accounts for the large increment in the mechanical response when compared with the normal twitch. In some instances the tension record showed two domes, separated by a trough—the classical record of veratrinized muscle. In those cases the spike potentials, recorded with the capacity-coupled amplifier at high gain, appeared as two bursts separated by a period of relative quiescence.

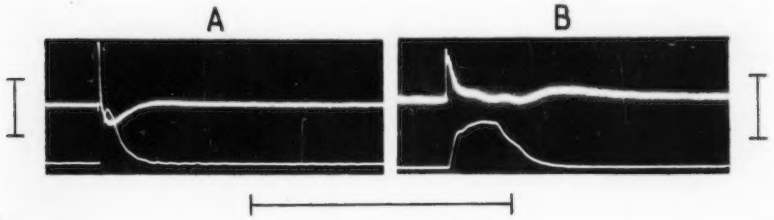

Fig. 4. Complex responses of muscle to single maximal indirect stimulation after veratrine. A, normal. B, after veratrine (2 mgm. per kgm.). Calibrations: 2 mv.; 200 grams; and 1 sec.

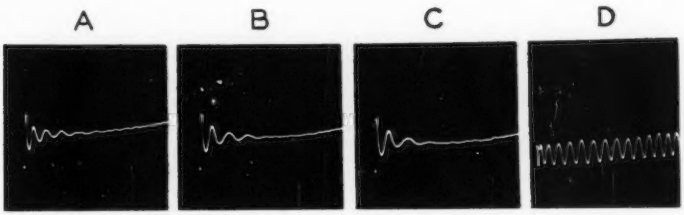

Fig. 5. Repetitive responses of muscle to single shock stimulation of the motor nerve after veratrine (1 mgm. per kgm.). The records show the early part of the 1st (A), 3rd (B) and 8th (C) responses in a series at 1 per sec. D, 200 cycles.

Repetitive stimulation at slow rates resulted in a decrease of the amplitude and duration of the successive mechanical responses; the bursts of spikes showed a parallel decline in frequency, amplitude and duration. Thus, the changes of the mechanical responses could be accounted for by corresponding variations in the spike potential records, and there was never any evidence of a contracture.

The successive spikes in a repetitive burst were at first relatively well synchronized, later they became small and irregular, indicating temporal dispersion of the discharging elements. The frequency of repetition could

be readily measured for the first 3 to 6 spikes in the burst. In a typical case (fig. 5) the interval between the successive spikes in the response to a single shock were as follows: 1st to 2nd, 4.5 msec.; 2nd to 3rd, 6; 3rd to 4th, 7; 4th to 5th, 7.5; and 5th to 6th, 7 msec. The corresponding frequencies declined, therefore, from 220 to 140 per sec. When the stimuli were applied at the rate of 1 per sec. the interval between the 1st and 2nd spikes for the successive responses was: 1st response, 4.5 msec.; 2nd, 5.2; 3rd, 5.3; 8th, 6.5 msec. The frequencies, therefore, showed again a decline.

The amplitude of the initial spike potential in response to single maximal nerve stimulation was increased up to 120 per cent of the normal by small and moderate doses of veratrine. Large doses resulted in a prolonged decrease, but an increase above normal was seen after recovery. The

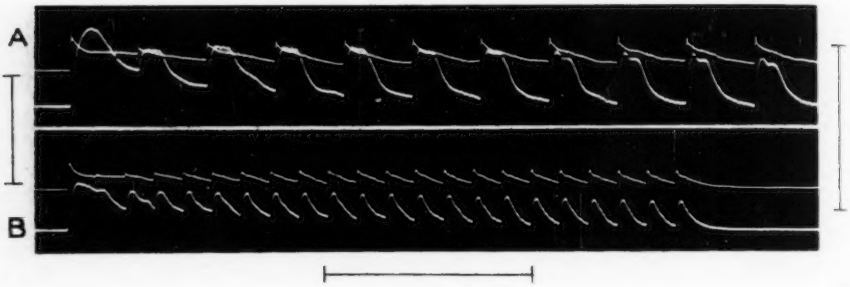

Fig. 6. Decrease followed by increase of the spike potential amplitude in response to repetitive indirect stimulation of muscle after veratrine (2 mgm. per kgm.). Records A and B show the effects of different frequencies. Note the absence of parallelism between the electric and the mechanical responses to the successive stimuli. Calibrations: 10 mv.; 500 grams; and 2 sec.

increase in amplitude was not due to a better synchronization of the several elements of the muscle, as judged by the duration of the spikes.

Repetitive stimulation of the nerve after veratrine resulted in characteristic changes of the amplitude of the initial spikes of the repetitive bursts set up by the stimuli. There was first a decrease and later an increase of the amplitude of these spikes. The mechanical effects corresponding to each stimulus did not show similar changes (fig. 6). It may be inferred, therefore, that the decrease of spike amplitude is not due to a decrease in the number of acting elements but indicates a diminution of the spike potential in each fiber.

Large doses of veratrine were by definition those which caused a marked decrease of the muscular responses. The effects were invariably reversible, although recovery took sometimes over an hour. Two different types of depression were observed. In some cases the decline of tension after the

injection, and the subsequent recovery, were quite parallel with the decline and recovery of the electric responses, and specifically of the initial spike potential amplitude. In other instances the decrease of tension was quite marked and prolonged, while the electric responses showed only a slight and transient reduction, or else after severe depression of both the electric and the mechanical phenomena the former recovered more rapidly than the latter (fig. 3). It was thus possible to record supernormal electric responses with subnormal mechanical effects (fig. 3, D and G). Such supernormal electric records showed not only a large initial spike potential and subsequent repetition, but also a large residual negativity.

An independence between the spike amplitude and the developed tension, on the one hand, and between the residual negativity and the tension, on the other, is thus revealed by veratrine. A relative independence between the spike potential and the slow negative waves was seen in the quicker recovery of the spike than of the residual negativity after a large dose of veratrine.

C. *The effects of yohimbine.* This drug augments the positive after-potential of nerve (Graham and Gasser, 1934). It was considered of interest to see whether it would similarly increase any of the positive components of the electromyogram. The results in 3 cats were inconsistent. In 2 cases yohimbine increased the positive wave in response to single nerve volleys, but in the third this wave was decreased and the negative waves became more prominent than normally. Again, in 2 cats negativity accumulated more than normally during tetanic stimulation, but in the other cat there was evidence of predominating positivity, rather than negativity, in these circumstances. The mechanical responses were not significantly modified by the drug.

Because of this inconsistency of effects the study of yohimbine was not pursued further. It is important, however, to emphasize that greatly increased residual negativity was seen during tetanic stimulation without any increase of the mechanical responses.

D. *The responses of denervated muscle to acetylcholine.* Denervated muscles respond to acetylcholine first by a contraction, then by a contracture (Brown, 1937; Rosenblueth and Luco, 1937). The period of contraction corresponds to a burst of spike potentials. In addition to these electric phenomena Schäffer and Licht (1926) have reported the appearance of a slow negative potential coincident with the development of tension.

Excluding from the following description the spikes which attend the contraction, one or more of the following slow potential changes were seen in the electrograms (see fig. 7). An initial positive excursion coincided with the beginning of contraction. It was promptly followed by a change of potential in the opposite direction, so that the muscle became

negative. No break in the electrogram corresponded with the transition from contraction to contracture. During and after the period of relaxation the muscle became increasingly negative. The peak of negativity, therefore, took place when the muscle had partially or totally relaxed. This negative potential then slowly subsided. The late negative wave was always present in the electrograms, while some of the other changes could be absent.

The initial positivity was less prominent when small (10 to 20γ) or large (100 to 150γ) doses of acetylcholine were injected than it was with intermediate doses. Successive injections with intervals of 30 to 90 sec. caused the appearance of progressively smaller electric responses, while the

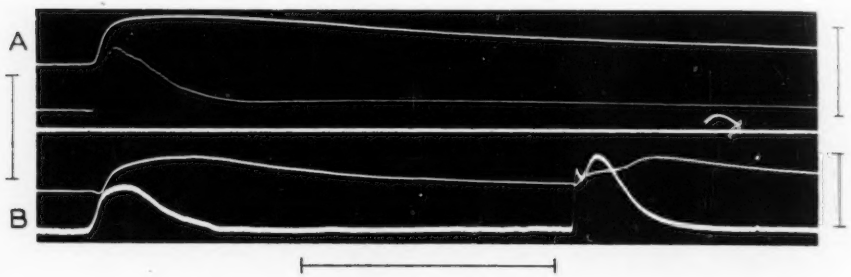

Fig. 7. Responses of denervated muscle to intra-arterial injections of acetylcholine.

A. Femoral nerve cut 13 days previously. Response to acetylcholine (160 γ).

B. Femoral nerve cut 12 days previously. Responses to 2 injections of acetylcholine (40 γ).

Calibrations: 10 mv.; 100 and 200 grams for A and B, respectively; 30 sec.

mechanical effects could either increase or decrease (fig. 7B). The positive component was sometimes emphasized by the later injections.

E. *The differences between sartorius and soleus.* Only quantitative differences were noted between the two muscles. Thus, while the average demarcation potential of sartorius was 14.5, that of soleus was 16 mv. The average maximal spike potential of sartorius was 3.4, that of soleus 7 mv. The slow potentials during and after repetitive stimulation of the two muscles varied similarly with changes of frequency, but the rate of stimulation at which any of the changes described previously took place (section A) was lower for soleus than for sartorius. The effects of veratrine (section B) were similar and equally striking on the two muscles (fig. 3).

It is frequently stated that neither sartorius nor soleus is composed exclusively of fast and slow muscle fibers, respectively, but that each is mixed, with the preponderance of one or the other type of muscular ele-

ments. The heterogeneity of the muscles was readily seen in the spike potentials, which could show 2 crests, and in the twitches, which could also show 2 components separated by a more or less well-defined trough. It was found, however, that both the fast and slow elements of sartorius are faster than the corresponding fast and slow components of soleus. Thus, the times for development of the two crests in the mechanograms of these muscles were 34 and 75 msec. for sartorius, and 45 and 105 msec. for soleus.

Discussion. That the spike potential of muscle is followed by slower electric phenomena was recognized by Bishop and Gilson (1927) and by Schaefer (1936). Schaefer referred to these slow components as afterpotentials. It is desirable to distinguish, if possible, the slow potentials associated with conduction from those associated with contraction. The conduction potentials include the spike potential and the afterpotentials; the contraction potentials would *a priori* be expected to include only relatively slow components of the electrogram. To complete the systematization of striated muscle potentials a third class of electric phenomena should be considered: the excitation potentials. To this class belong the end-plate potentials studied by Göpfert and Schaefer (1937), by Eccles and O'Connor (1939) and by Feng (1940), since these potentials are not associated with either conduction or contraction.

The similarity between the conducting mechanism in muscle and that in nerve is supported by the similar action of veratrine upon the two structures. Thus, the responses of nerve to single shock stimulation are repetitive after veratrine (Dun and Feng, 1940; Acheson and Rosenblueth, 1941). The repetitive responses of sartorius in figure 5 could be due to repetition of the motor nerve impulses. Bacq and Brown (1937), and Feng (1938) have shown, however, that curarized and veratrinized muscles also respond repetitively to single-shock direct stimulation.

The initial spike amplitude in the responses of veratrinized nerve to repetitive stimulation decreases typically for a brief period of time and later increases (Acheson and Rosenblueth, 1941). Similarly the initial spike amplitude of veratrinized muscles stimulated repetitively shows an early decline and a late rise (fig. 6).

The negative afterpotential of nerve is augmented by veratrine (Graham, 1930). The residual negativity of muscle is in turn greatly increased after injections of the drug (figs. 3, 4 and 6). It may be inferred that this augmented negativity is due to a large negative afterpotential—i.e., that it represents a conduction, not a contraction potential. This inference is supported by the independent variations of the amplitude and the time course of the slow negative potentials and of the mechanical responses of veratrinized (fig. 3) and yohimbinized (p. 731) muscles.

Several of the positive potentials recorded did not show any temporal correlation with the changes of tension in the muscle during and after

contraction (sections A and B). For this reason and in analogy with the phenomena in nerve, it is likely that some of these positive changes may be muscular conduction afterpotentials. As stated in section C, the test of this inference by yohimbine failed to yield a consistent answer. The irregular results obtained with this drug may be accounted for by the assumption that yohimbine augments not only the positive, but also the negative afterpotential of muscle.

None of the potential changes described was invariably correlated in time course with the mechanical response. However, when the development of tension coincided with an electric deviation, the potential was relatively positive. Thus, the early positive swing during high frequency simulation of normal muscles (fig. 2G) had a time course approximately parallel with that of the initial rise of tension. It is tentatively inferred, therefore, that at least one of the contraction potentials is a positive potential. This inference may be surprising, since activity has been traditionally associated with negativity. However, there is no *a priori* reason for the association of any specific electrical sign with the physicochemical changes attending contraction.

The muscular changes associated with contraction are not limited to the development of tension but include also recovery processes. It is likely that such processes include physicochemical changes with electric manifestations. It is possible, for instance, that the prolonged delayed negative wave elicited by acetylcholine in denervated muscles may correspond to such recovery. This wave might be due, however, to a prolonged depolarization of the muscle. A similar negativity was seen in normal muscles after injections of KCl, with little or no preceding contraction. A more detailed systematization of muscle potentials requires further data. With the present evidence it appears that the contraction potentials are largely masked by the conduction phenomenon.

The relations existing between the spike potential and the mechanical response in striated muscle are still undetermined. While Brücke (1908) and Beritoff (1924) stressed the independent variation of the two phenomena in fatigue, Fulton (1926) and Davis and Davis (1932) suggested that the spike potential is the agent which directly releases the energy changes which attend contraction. The present data indicate that there is a large degree of independence between the spike potential and the mechanical effects. Thus, the mechanical responses may be equal when the spike potential amplitude varies considerably upon repetitive stimulation after veratrine (fig. 6). Furthermore, during the period of recovery after injection of a large dose of this drug, large spike potentials may be followed by minimal contractile responses (fig. 3). It is concluded, therefore, that although the spike potential may directly or indirectly activate the contractile system, the magnitude of the mechanical effect is independent of that of the electrical phenomenon.

SUMMARY

The electric responses of circulated cat's muscles (mainly sartorius) were recorded with direct-coupled amplification together with the mechanical effects of indirect stimulation.

The electrogram of normal muscles is complex; a spike potential is followed by slow potential changes (fig. 1). Repetitive stimulation results in summation of some of the slow components (fig. 2). Veratrine increases the negative slow components of the electrogram (figs. 3 and 4) in addition to other striking effects (figs. 5 and 6; section B). Yohimbine may similarly increase the negative residual potentials without any significant change of the mechanical reactions (section C). The electric responses of denervated muscles to injections of acetylcholine include several slow components (fig. 7).

The following systematization of muscle potentials is suggested: excitation potentials, which precede conduction (e.g., the end-plate potential); conduction potentials (the spike and the afterpotentials); contraction potentials (those associated with the development of tension and with the corresponding recovery processes).

None of the potentials recorded was invariably correlated in time with contraction. When such a correlation appeared the potential was relatively positive. It is inferred that one of the contraction potentials may be positive. An independent variation of the residual negativity and of contraction is produced by veratrine (fig. 3). It is inferred that this negativity denotes an afterpotential. The spike potential amplitude and tension may also vary independently (figs. 3 and 6).

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SOME EFFECTS OF VERATRINE UPON CIRCULATED MAMMALIAN NERVES

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The original purpose of this study was to compare the properties of nerves, muscles and neuromuscular junctions as revealed by the effects of certain drugs, among which was veratrine. It soon became apparent, however, that the information available on the action of veratrine upon mammalian nerves was not sufficient for that comparison. The present paper deals with some of the effects of veratrine on the A fibers of circulated cat nerves.

METHOD. In cats under dial anesthesia (Ciba, 0.75 cc. per kgm., intraperitoneally) the sciatic nerve on one side was cut at its emergence from the pelvis. The peroneal nerve was cut near the head of the fibula and the central end was dissected free for about 3 cm. One or two pairs of shielded silver-wire electrodes were placed for stimulation toward the pelvic end of the segment of the peroneal isolated by the cuts. In order to prevent muscular responses by spread of the stimuli, the popliteal and the nerves to the hamstring muscles were cut.

Recording electrodes of the Sherrington type (shielded by glass tubing) were applied to the dissected peripheral end of the peroneal. Two large chlorided silver wires were sufficiently impolarizable for accurate recording of the nerve potentials. The stimuli were condenser discharges rendered diphasic by passage through a transformer.

Care was taken to preserve the blood vessels of the nerve. Intra-arterial injections were made into the abdominal aorta through a cannula tied in the inferior mesenteric artery. That the nerve was adequately supplied with blood was shown by the prompt effect obtained upon injection of veratrine (see fig. 6). The upper parts of the animal gave much less evidence of veratrine poisoning than the area supplied by the lower abdominal aorta. The heart remained strong, and adequate respiration continued.

The electric responses were recorded from a cathode-ray oscillograph. For the investigation of the slow components of the responses and their correlation with spikes, a five-stage direct-coupled amplifier was used. The more rapid components were more conveniently studied with the aid of a five-stage capacity-coupled amplifier.

RESULTS. A. *Demarcation potential.* The records were always taken from an intact to a crushed region of the nerve. Changes of the demarcation potential could therefore be readily followed in all the experiments in which the direct-coupled amplifier was used.

Bishop (1932) observed a decrease of the demarcation potential when a region of a nerve was treated by veratrine. A decrease was frequently seen in the present study. On the other hand, an increase, instead of a decrease, was also occasionally present. The conditions which determine the sign of the change were not studied. For present purposes it is sufficient to state that all the effects to be described below could occur when the demarcation potential had been increased or decreased, or was practically unchanged by the dose of veratrine administered.

B. *Conduction velocity.* The conduction velocity of the fastest A fibers was calculated from the stimulus-response interval in the usual manner. The sources of error inherent to this method are well known. In order to minimize the error due to spread of the stimulus, submaximal shocks were employed, which activated about 50 per cent of the A fibers in the nerve.

Relatively small doses of veratrine (e.g., 0.5 to 1 mgm. per kgm.) resulted as a rule in no significant change of conduction velocity. Larger doses caused usually a marked decrease of conduction velocity of both the α and β elements in the nerve. Thus, in a typical instance, while an initial injection of veratrine, 1 mgm. per kgm., produced no slowing of conduction, a further similar injection made 10 min. later reduced the velocity of the fastest A fibers from 100 m. per sec. to 73 m. per sec.

When the nerve was stimulated repetitively at frequencies of 1 to 60 per sec. after injections of veratrine, all responses after the first one took place before subsidence of the negative afterpotential corresponding to the preceding response (see section D). Indeed, in these conditions the nerve impulses often occurred during the period of increased electrical excitability following the preceding impulse, that is, during the period of supernormality. Whether or not veratrine had slowed the conduction velocity of the resting nerve there was never any evidence of an increase in the rate of conduction of impulses during the supernormal period. These observations agree with those of Graham and Lorente de Nó (1938) on normal circulated mammalian A fibers.

C. *Repetitive responses.* Veratrine causes nerves to discharge repetitively in response to single brief submaximal or just maximal shocks (see Gasser, Richards and Grundfest, 1938; Dun and Feng, 1940). The first few repetitive responses were fairly synchronized and occurred at rates as high as 660 per sec. (fig. 1). They then became progressively less synchronized and gave way to a continuous, gradually decreasing, asynchronous firing, which might last as long as 25 sec. after a single stimulus (fig. 4).

The degree of repetition could be judged either by the rate and amplitude

of the early synchronized responses or by the amplitude of the later, asynchronous discharge. In the latter case, a large amplitude, that is, a high average spike height, was taken to indicate frequent random synchronization of large numbers of elements firing at high frequencies. The

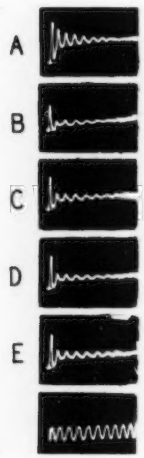

Fig. 1

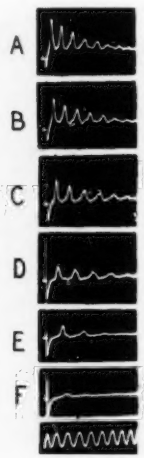

Fig. 2

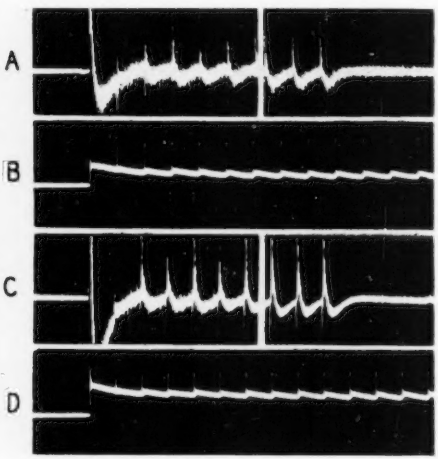

Fig. 3

Fig. 1. Early synchronized repetitive spikes and augmented negative afterpotential of circulated peroneal nerve after veratrine (1 mgm. per kgm.). A to E, first 5 responses to maximal shocks at a frequency of 0.7 per sec., demonstrating the decline of rate and magnitude of repetitive responses after the first shock. In this and subsequent figures showing sweeps, stimulus occurs at extreme left. Calibration at bottom, 500 cycles. Direct-coupled amplifier.

Fig. 2. Influence of frequency of stimulation on rate of repetitive responses after veratrine (4 mgm. per kgm.). Each sweep represents the response to a single maximal shock several seconds after the beginning of a series at one of the following frequencies: A, 0.38; B, 0.82; C, 1.8; D, 3.2; E, 5.6; and F, 9.0 per sec. Calibration, 500 cycles. Capacity-coupled amplifier.

Fig. 3. Effect of veratrine on repetition and negative after-potential. Stimulation at 8 per sec. A and B, after a dose of 2.4 mgm. per kgm.; C and D, after an additional dose of 5 mgm. per kgm. A and C, condenser-coupled amplifier at high gain, to show asynchronous repetition. B and D, direct-coupled amplifier at lower gain, to show negative after-potentials. The initial spikes were larger than appears in all the records.

gradual decline of repetition rendered the duration of the response an inaccurate measure of repetition.

With increasing doses of veratrine the two criteria mentioned above generally revealed a greater degree of repetition. Figure 5 illustrates the increase of both the rate and the amplitude of the early synchronized responses with successive doses of the drug. A similar increase with dose

was found when the amplitude of the asynchronous phase of the response was studied. Large doses could bring about a decrease of the repetition (fig. 3A and C).

When, beginning with barely liminal stimuli, the shocks were progressively intensified the results were as follows. Repetitive responses were elicited even by weak shocks which activated only a fraction of the α fibers. As the shocks were strengthened the degree of repetition increased with respect to the amplitude of the irregular spikes recorded. Shocks 3 or 4 times stronger than those maximal for the A and B fibers of the nerves did not cause any significant increase of repetition over that elicited by just maximal stimuli. It may be concluded, therefore, that here the degree of repetition is not a function of the intensity of shock used, but of the number of fibers activated.

The description has dealt thus far with the results of single shocks. If a second stimulus was delivered before the repetitive response to the first shock had subsided—in other words, if repetitive stimulation at various frequencies was used—the asynchronous phase of the response showed the following changes. The additional stimuli, after the first, caused an increase of the discharges. As a rule the first shock in a train caused the greatest effects; additional shocks produced less repetition. Occasionally, however, the second or third shock resulted in more repetition than that from the first. The greater the frequency of stimulation, the less the increment of response contributed by each additional shock. Even after prolonged repetitive stimulation, however, each shock still elicited a repetitive discharge, if the frequency was moderate. Thus, in one observation after 20 sec. stimulation at the rate of 5 per sec. repetitive bursts were still present. The subsidence of the repetitive discharges after the last shock in a train of stimuli took place sooner than when a single stimulus was applied.

The influence of the frequency of stimulation on the degree of repetitiveness elicited per shock could also be seen as a change in the rate and amplitude of the synchronous volleys immediately following each stimulus. Figure 1 shows these early repetitive responses to a series of stimuli at 0.7 per sec. The first stimulus produced a response in which repetitive firing began at a rate of 660 per sec. In response to the third stimulus the rate of repetition was 530 per sec., and in the subsequent responses the same rate was maintained. By applying different frequencies it was found that the level of this steady state is a function of the frequency of stimulation. Figure 2 exemplifies this relation. With increasing frequencies of stimulation the rate and amplitude of repetition decreased. When stimulation rates of 1 per sec. or less were employed, the repetition was essentially like that which follows a single shock in the rested nerve.

D. *The negative afterpotential.* As shown by Graham (1930) veratrine

greatly increases the negative afterpotential of nerve. In figure 6 is shown the prompt increase of negative afterpotential following injection of veratrine (0.5 mgm. per kgm.) in a typical observation. Negative afterpotentials with peak values as high as 2 to 4 mv. were recorded. They some-

Fig. 4. Prolonged repetitive discharge in response to a single maximal shock after veratrine (3.5 mgm. per kgm.). Capacity-coupled amplifier, high gain. The initial spike went off the tube. The successive strips show the asynchronous discharges at the beginning of the response and 5, 10 and 15 sec. later. The irregularities in the last record as compared with the initial background before stimulation indicate that the nerve was still active. Time calibration: 1 sec.

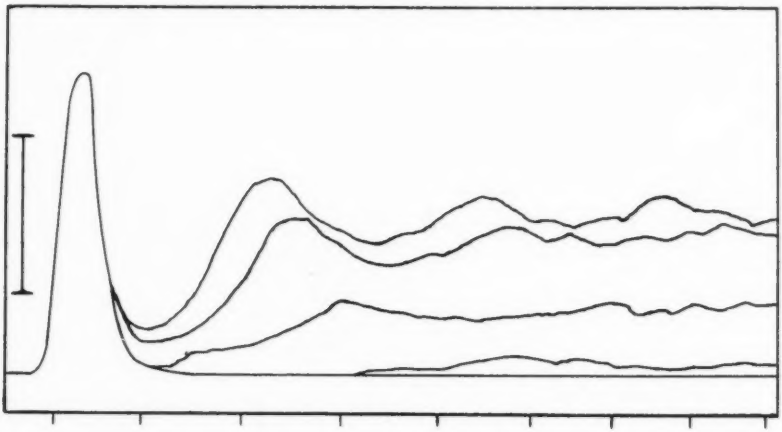

Fig. 5. Influence of dose of veratrine on repetition. Superimposed tracings from the original film. Responses to single maximal shocks. Lowest record: normal control. The progressively ascending records correspond to responses after veratrine, 1, 2, 3 and 4 mgm. per kgm. Time: 1 msec. intervals. Voltage calibration: 1 mv.

times attained 60 per cent of the magnitude of the conducted spike and were still measurable more than 12 sec. after the stimulus. Average figures after 2 to 3 mgm. per kgm. of veratrine were as follows: spike magnitude (7 to 8 cm. conduction), 3 to 3.5 mv.; peak of negative afterpotential, 1.5 to 2 mv.; duration of negative afterpotential, more than 6 sec.

When repeated small doses of veratrine were injected at intervals of 5 to 30 min. it was seen that the amplitude of the negative afterpotential is directly related to the dose. In figure 7 are illustrated responses to single shocks after various doses of the drug. The amplitude of the peak of negativity gave a sigmoid curve when plotted against the dose of veratrine administered. It is interesting to note that after a certain amount of the drug was present, an additional injection could yield a significant further

Fig. 6. Prompt effect of intra-arterial injection of veratrine as revealed by negative afterpotential of peroneal nerve stimulated maximally at 0.8 per sec. Injection started 2 sec. before beginning of record; arrow marks end of injection. The excursions correspond to the negative afterpotential; the spikes are too fine to be seen.

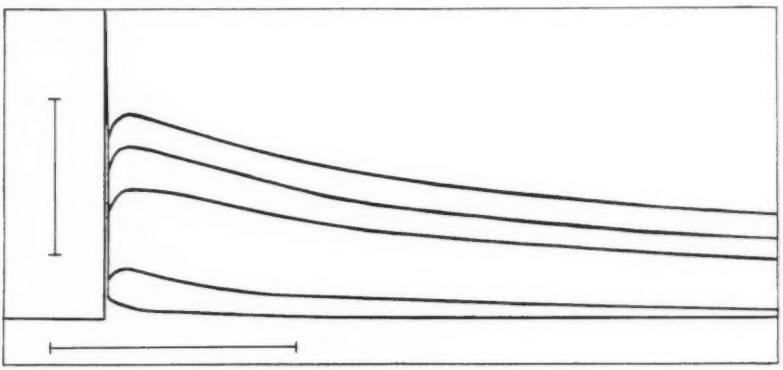

Fig. 7. Influence of dose of veratrine on negative afterpotential. Superimposed tracings from the original film. Responses to single maximal shocks. The progressively ascending records correspond to 0.2, 1.4, 2.4, 4.4, and 5.4 mgm. per kgm. of veratrine. Time calibration: 0.1 sec. Voltage calibration: 1 mv.

increase of the negative afterpotential, whereas the degree of repetition caused by a single stimulus was reduced (fig. 3).

As revealed by fast records, the peak of the negative afterpotential occurred usually some milliseconds after the first spike potential had finished (fig. 5). Doses of veratrine which caused a significant increase of the negative afterpotential caused likewise the appearance of repetitive discharges. That a delayed maximum of residual negativity may develop without any detectable repetition has been shown, however, by Gasser and Graham (1931).

As was shown above for the degree of repetition, the amplitude and duration of the negative afterpotential did not increase when the stimuli were intensified beyond maximality. It may be concluded that the negative afterpotential is elicited by the spike response, not directly by the stimulus (see Gasser and Erlanger, 1930).

Repetitive stimulation at frequencies of 1 to 120 per sec. resulted in a summation of the negative afterpotentials corresponding to the successive responses (fig. 8). As a rule the highest residual negativity developed for any train of stimuli was that present at the peak of the response to the first shock. Only rarely did relatively high frequencies of stimulation (e.g., 60 per sec.) produce, after a few shocks, more negativity than that attained

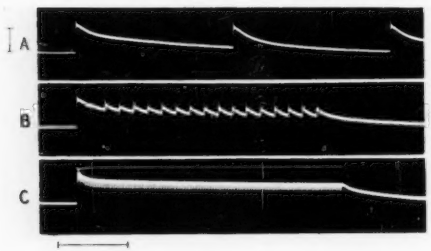

Fig. 8

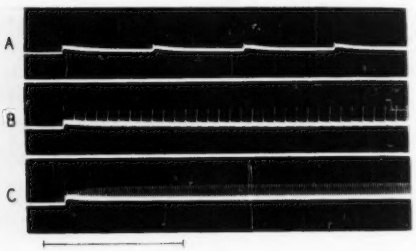

Fig. 9

Fig. 8. Negative afterpotentials in response to maximal stimulation at various frequencies after veratrine (2 mgm. per kgm.). Frequencies: A, 0.45; B, 4.1; and C, 60 per sec. Direct-coupled amplifier. Voltage calibration: 2 mv. Time calibration: 1 sec. The spikes were larger than appear in the records.

Fig. 9. Changes in spike magnitude in response to maximal stimulation at various frequencies after veratrine (2 mgm. per kgm.). Frequencies: A, 1.6; B, 13; C, 60 per sec. Direct-coupled amplifier. Voltage calibration: 5 mv. Time calibration: 1 sec.

in response to a single stimulus. With increasing frequencies of stimulation the additional residual negativity contributed by each shock decreased.

E. *Spike magnitude.* Moderate doses of veratrine (e.g., 1 to 2 mgm. per kgm.) usually caused no significant change or a slight increase of the spike magnitude in response to a supramaximal stimulus. Further injections resulted in a decrease of the amplitude of the spike potential. Such a decrease could be due to a change of the response in each of the axons of the nerve, or it could be produced by the dropping out of some axons because of impaired conduction, the remaining fibers retaining a full-sized spike. It will be shown below that veratrine may indeed prevent conduction in nerves. When this occurred the nerve responses returned within a few minutes to slightly less than the spike magnitude prevailing before the injection. The decrease in amplitude under consideration in this section,

on the other hand, endured, practically unabated, for over an hour. It is likely, therefore, that veratrine can produce a decrease of the spike-potential amplitude of A fibers.

Repetitive stimulation after veratrine resulted usually in a characteristic sequence of the spike potentials recorded. The responses to successive shocks in a train first decreased in amplitude to reach a minimum at about 15 to 50 msec. after the beginning of the series of stimuli. The spikes grew thereafter, remaining as a rule lower than the first maximal spike even if the stimuli were delivered for 5 to 30 sec. The changes were not due to a decrease of the excitability of the nerve fibers, for the stimuli could be greatly intensified beyond maximality with no change in the results. With frequencies of 0.5 to 200 per sec. the final level, e.g., after 5 sec. stimulation, was lower for fast than for slow rates of stimulation. With higher frequencies (e.g., 300 to 500 per sec.) the results were complex and their study was not pursued.

Figure 9 illustrates these changes in spike magnitude. It is evident that the reduction of amplitude for all the shocks subsequent to the first is present whether the spikes be measured from the baseline from which they depart or from the original baseline prevailing before the repetitive series of stimuli was started.

In all the cases in which this reduction of response was observed the shocks were applied at a time when the nerves were firing repetitively as a consequence of preceding stimuli. It is probable, therefore, that some of the fibers in the nerve should have been refractory at the time of application of the later stimuli. A reduction in the amplitude of the recorded spikes could then be due to a decrease in the number of responding elements. It is impossible to evaluate accurately the rôle of this refractory condition of some fibers in the records obtained, and hence to decide whether or not an additional factor was at play. The following observations favor the interpretation that the phenomenon is not due exclusively to a decrease of the number of fibers activated by stimuli subsequent to the first in a series. With relatively small doses of veratrine a marked degree of repetition was seen in several animals with only a slight drop of the ceiling of the spikes elicited by trains of stimuli at various frequencies. Further injections of veratrine resulted occasionally in a reduction of the repetitive after-discharge with a greater decrease of spike amplitude during repetitive stimulation (fig. 3).

In two cats the whole sciatic was stimulated. The mechanical responses of the Achilles tendon muscles were recorded on a kymograph and the electric responses of the peroneal nerve were led to the amplifier and oscillograph as usual. Figure 10 illustrates the records obtained after veratrine (3 mgm. per kgm.). It is clear that the reduction of nerve electric response to successive stimuli is not attended by a parallel reduction in the mechano-

gram, as would have taken place had there been fewer nerve fibers activated by the stimuli after the first shock. It is concluded, therefore, that the decrease of initial spike amplitude denotes a diminution of the spike potential of the individual fibers.

F. *Alternation.* At certain frequencies of stimulation the responses of veratrinized nerve often showed alternation. Typically the responses to the third, fifth, seventh and ninth shocks in a series were larger than those

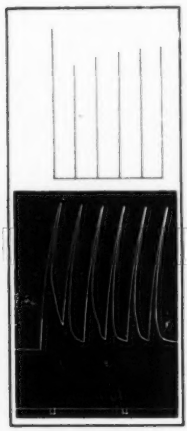

Fig. 10

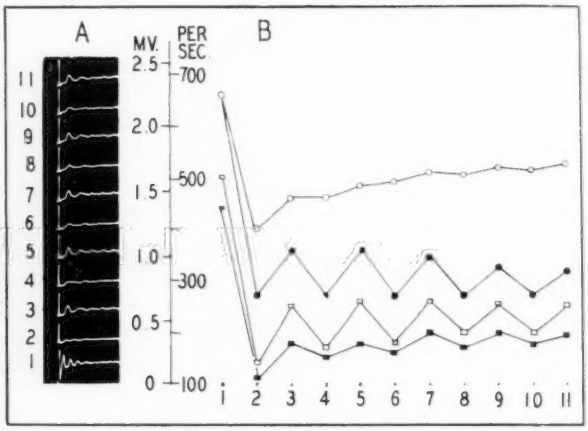

Fig. 11

Fig. 10. Independence of nerve spike amplitude and of muscular responses after veratrine (4 mgm. per kgm.). Maximal stimulation of the sciatic nerve at the rate of 0.7 per sec. Lower record: mechanogram from Achilles-tendon muscles (time signal: 5 sec.). Upper tracing: relative magnitude of the initial spikes recorded monophasically from the peroneal nerve.

Fig. 11. Alternate degree of response to repetitive stimulation at 7 per sec. after veratrine (4.4 mgm. per kgm.). A. Responses to first 11 shocks in a series. Direct-coupled amplifier. B. Measurements of several features of the responses in A. The numbers in the abscissae correspond to the successive shocks. Circles: magnitude of initial spike. Dots: rate of repetition (calculated from first 2 or 3 intervals). White squares: magnitude of second spike. Black squares: increment of negative afterpotential.

to the even-numbered shocks. Occasionally every third or fourth shock produced a larger response. While alternation usually decreased and became unrecognizable after the seventh or ninth shock, in some instances it persisted through as many as twenty responses.

Several features of the responses participated in the alternation. Figure 11A shows the early part of the responses to the first eleven maximal shocks delivered to the nerve at a rate of 7 per sec. In figure 11B the data for the response to each shock are plotted in columns corresponding to the order

of shocks. The most striking alternation occurred in the magnitude (white squares) and rate (dots) of the synchronized repetitive spikes of the responses. There was no alternation of the individual spikes within each repetitive train; the train as a whole grew alternately larger and smaller in response to successive shocks. The increment of negative afterpotential contributed by each shock (black squares) also waxed and waned strikingly. Less obvious, in some instances not demonstrable, was alternation in the magnitude of the initial spikes following the stimuli (circles).

Frequency of stimulation was critical for the occurrence of alternation. The phenomenon rarely occurred at rates lower than 5 or higher than 10 per sec. If maximal stimulation was gradually accelerated from 1 to 15 per sec. alternation appeared suddenly at some frequency within that range and then vanished at a higher frequency. With this procedure the magnitude and rate of the early synchronized repetitive spikes of the larger responses during alternation were greater than those of non-alternating responses at frequencies just below the range of alternation.

The appearance and degree of alternation were not affected by increasing the strength of the stimuli up to 3 times maximal; they were not influenced by the dose of veratrine (from 0.5 to 6.5 mgm. per kgm.). There was no correlation between the degree of alternation and the changes of initial spike magnitude on repetitive stimulation illustrated in figure 9.

G. *Electrical excitability.* An increase of the resting electrical excitability of nerves after veratrinization has been reported by several observers (see, for references, Graham, 1934). Increased excitability was commonly seen in the present study soon after an injection of the drug. This stage, however, was followed within a few minutes by a prolonged decrease so that the resting electrical excitability was less than before the injection.

When several small doses (e.g., 0.2 to 0.5 mgm. per kgm.) were injected with intervals of 10 to 30 min. each successive dose resulted, as a rule, in the sequence of changes of excitability mentioned above, first a transitory increase and then an enduring decrease. Since the successive injections were often given at intervals of time which insured cumulative effects, it is likely that the period of increased excitability does not correspond to a small concentration of the drug in the nerves but rather to the penetration of the poison into the nerve.

Whereas the period of increased excitability was too brief for a detailed study of the strength-duration curve, that of decreased excitability could be studied at leisure. The voltage-capacity curve was found to shift upward and to the left. The voltage parameter increased markedly, while the time parameter decreased slightly.

In some observations the nerve was stimulated by fairly long (17 msec.) direct current pulses. In normal nerves the lowest threshold for stimulation is to the make of the current, and a response to the break is obtained

only with 2 to 5 times stronger currents. After veratrinization the response to the break was obtained with the same intensity as, or even with weaker currents than, those necessary for responses to the make. This striking change in the relative thresholds for stimulation with the make and the break may explain effects seen occasionally using condenser discharges as stimuli. When the cathode was proximal to the recording electrodes and the voltage was progressively increased the first response detected had a long latency, corresponding to conduction from the distal anode. With stronger stimuli an additional earlier spike appeared with a latency proper to conduction from the cathode. Further intensification resulted in an increase of the early response and a decrease of the later spike. Finally only a maximal A spike from the cathode was seen.

The changes of excitability resulting from conditioning the nerve by a maximal stimulus were studied as follows. Two pairs of stimulating electrodes were applied. The pair distal to the recording electrodes was used for the conditioning stimulus, and the proximal pair delivered the single or repetitive submaximal test stimuli. A decrease or an increase of excitability after the maximal conditioning stimulus was indicated by a decrease or an increase of the amplitude of the responses to the test stimuli.

In some of the animals studied veratrinization did not result in a period of supernormality—i.e., at no time after a conditioning stimulus was there any evidence of increased electrical excitablity; indeed, the obvious result of veratrine could be a prominent subnormality prevailing for over a second after the conditioning shock. This absence of supernormality was seen in nerves in which the dose of veratrine injected had caused marked repetition and increase of the negative afterpotential. These results agree with the observations made by Graham and Gasser (1931).

In other cases the conditioning volley was followed by supernormality which disappeared after about 0.1 sec. Finally, in other instances an initial subnormality was followed after 0.05 to 0.15 sec. by a later more prolonged supernormality. In all cases a large and long negative afterpotential was present. When an apparent subnormality was present the minimal responses coincided roughly with the peak of the negative afterpotential of the conditioning volley and with the minimum of the decrease of spike magnitude when a series of maximal stimuli were applied (section E).

The results are complicated, and their interpretation made consequently difficult, by the two following factors. First, an approximate estimation of the number of nerve fibers activated by a submaximal test stimulus can be made by comparison with the responses elicited before conditioning only if the spike amplitude per fiber remains constant. It was shown above, however, that the spike magnitude varies during repetitive stimulation after veratrine (fig. 9).

The second complicating factor for the interpretation of the observations on changes of electrical excitability with repetitive test stimuli lies in the phenomenon of recruitment. As pointed out by Gasser (1938) trains of subliminal stimuli are able to recruit fibers over series of several shocks, so that the responses become progressively larger as stimulation is continued. Veratrine favors, as a rule, the appearance of recruitment. The observations of Gasser were fully confirmed. The first complicating factor—a change of spike magnitude—was present in all the observations, whether single or repetitive test shocks were employed. The factor of recruitment was especially significant when the test stimuli were applied continuously at relatively fast rates (1 to 60 per sec.).

H. *Abolition of nerve responses.* Doses of veratrine of 2 to 5 mgm. per kgm. greatly diminished or canceled entirely any recordable electric responses. The effect was reversible; within a few seconds to several minutes, spike potentials could again be detected, their amplitude increased and a complete recovery was soon reached. Further injections could again lead to new reversible cancellation of the responses.

In 5 animals the whole sciatic nerve was stimulated. The electric responses of the peroneal or the popliteal nerve and the mechanical responses of the Achilles tendon muscles were recorded. In 4 of these 5 cats a decrease or an abolition of the muscular responses coincid/ d rigorously with a decrease or an abolition of the nerve responses. In the exceptional animal the muscular responses were markedly and reversibly reduced by doses of veratrine which did not significantly decrease the amplitude of the nerve spike potentials. It may be inferred that in the conditions of these experiments failure of muscular responses after large doses of veratrine is usually due to a toxic effect on the motor nerve fibers.

Discussion. The appearance of repetitive responses after veratrine was coincident with an increase of the negative afterpotential. The latter is probably a manifestation of a process elicited by the spike response, not directly by the stimulus. Thus the conduction velocity of the negative afterpotential is the same as that of the spike potential. Furthermore, the amplitude and time course of the negative afterpotential do not depend on the intensity of the stimuli (p. 742). It is likely, therefore, that the additional spikes in a repetitive burst add to the residual negativity caused by the first spike in the response. That veratrine increases the negative afterpotential resulting from a single spike is shown, however, by the greater residual negativity of veratrinized nerves as compared to normal nerves when stimulated repetitively even at high frequencies. It is also shown by the observations on excised nerves, in which an increase of the negative afterpotential is the rule and repetition only exceptional (see Graham and Gasser, 1931; Gasser, Richards and Grundfest, 1938).

The results of repetitive stimulation (fig. 8) support Gasser's (1937)

conclusion that the negative afterpotential is capable of summation. The maximal residual negativity occurred as a rule shortly after the first spike and further stimuli had only slight additional effects. The suggestion emerges that the process responsible for the negative afterpotential is limited and that a period of recovery is necessary after a response before a full-sized development of the process may be renewed. The existence of a recovery period for the process underlying the negative afterpotential does not support the view that the residual negativity is due to the metabolites of nerve conduction (see Gasser, 1937, for a discussion of the interpretation of nerve afterpotentials).

The frequency and duration of the repetitive burst of impulses elicited by a single stimulus after veratrine does not depend upon the intensity of the stimulus (p. 739). It is likely, therefore, that the burst is a consequence of the first spike. As in the case of the negative afterpotential, successive shocks in a series add only slightly to the preëxisting repetitive responses. An argument similar to that used above suggests that the process underlying repetition is limited and requires a recovery period.

Repetitive discharges of mammalian A fibers in response to a single brief shock may be seen in other conditions: alkalinity, asphyxia, low Ca (Lehmann, 1937a, b, c). That the mechanism of repetition after veratrine is probably different from that which corresponds to those conditions is suggested by the two following considerations. In the cases studied by Lehmann the repetition was mainly an exaggeration of spontaneous continuous discharges taking place without stimulation; furthermore, the resting electrical excitability of the nerves was greater than normal. After veratrine, on the other hand, the resting electrical excitability may be less than normal (p. 745) and there is no evidence of any spontaneous discharges outside the periods of stimulation.

Gasser and Grundfest (1936) found a good correlation between the degree of spontaneous repetitive discharge of cut phrenic nerves and the supernormal excitability during the periods of negative afterpotential. Such a correlation was also present in Lehmann's (*loc. cit.*) observations. It is likely that supernormality, when present, may favor repetition of responses after veratrine. Repetition was seen, however, in nerves in which there was no evidence of supernormal excitability at any time after a conditioning shock (p. 746). Since in these nerves, as well as in those which did show supernormality, the resting electrical excitability was decreased by veratrine, it is clear that repetitive responses may occur despite a depressed electrical excitability.

An increased excitability of nerve would probably promote repetition of response whatever the mechanism responsible for that repetition. It would, on the other hand, be an indispensable factor only if repetition were due to a continuous subliminal stimulation. An alternative assumption

is that nerve, like the heart, has the intrinsic ability to discharge rhythmically. Veratrine may favor the manifestation of this ability. The long enduring rhythmic discharges could then be due to any of the long enduring processes which follow the first response, e.g., the process which underlies the appearance of the negative afterpotential. The analogy with the heart is supported by the well-known fact that a quiescent heart or strip of cardiac muscle may start beating rhythmically after application of a single stimulus.

As mentioned in section E, the initial spikes in response to successive shocks after veratrine show a characteristic decline of amplitude followed by a later increase. Some of the factors which complicate the interpretation of the phenomenon have been stated (p. 743). Since the first shock causes the appearance of a repetitive burst the subsequent stimuli may find some of the fibers refractory. Furthermore, increased temporal dispersion of the recorded response due to slowed conduction during the relatively refractory period would result in a decrease of the magnitude. The observations in which the muscular responses were recorded together with the nerve potentials (fig. 10) strongly support, however, the inference that the early decrease of the recorded spikes is due at least in part to a diminution of the spike amplitude of each fiber.

The decrease of the spikes occurs at the time when the negative afterpotential is approximately at its peak. It is tempting to infer a correlation from this parallelism. Certain observations, however, suggest an additional mechanism for spike depression. Thus, in some experiments a given dose of veratrine determined the appearance of a large negative afterpotential without any significant early decline of the spikes. This additional factor may be the rate of repetition of the responding fibers. The assumption that the amplitude of the spike potential is inversely proportional to the frequency of discharge accounts both for the early decline of the responses—since many fibers would be then discharging at a rapid rate—and for the later rise, when the rate of repetition would probably be slower. This assumption agrees also with the decline of spike amplitude observed upon rapid stimulation of normal nerves.

Alternation (fig. 11) could be due to variability either in the number of fibers activated by successive stimuli or in the responses of a constant number of fibers—i.e., alternation of individual fibers. Since the initial spike amplitude alternated only slightly as compared to the marked changes in the other features of the responses, and since alternation was independent of the intensity of stimulation (p. 745), it is concluded that alternation may occur in individual fibers. The existence of a critical range of frequencies for alternation suggests that the phenomenon is due to a cyclical process with a period of 100 to 200 msec. The nature of this process is obscure.

It has been frequently assumed that veratrine has a "curarizing" action upon neuromuscular systems—i.e., that, like curare, it stops transmission of motor nerve impulses, the nerve and the muscle remaining independently excitable and functional. The observations in section H render this assumption questionable (see also Boehm, 1920). In 4 of the 5 animals in which both nervous and muscular responses were recorded, a failure of the muscular reaction took place only when the nerve was obviously unresponsive. Such results are quite different from those produced by curare. Even in the one animal in which a decrease of muscular contractions was seen while the nerve responses were unimpaired it is not certain that the failure was one of transmission. It is possible that the muscle may have become correspondingly unresponsive.

A relatively simple hypothesis to account for all the striking changes which veratrine causes in nerve might be elaborated if a consistent correlation of these changes were present. A broad correlation of some of the changes is apparent upon superficial examination of the data. Thus, as a rule, a large negative afterpotential coincided with marked repetition of responses, with a supernormal electrical excitability after a conditioning shock, and with a decrease of spike magnitude upon repetitive maximal stimulation. Indeed, the time course of the four effects was roughly similar.

A closer study, however, reveals important discrepancies in this correlation. Thus, large doses of veratrine may lead to large negative afterpotentials with only slight repetition (fig. 3). Similarly, marked residual negativity may take place without supernormal excitability (p. 746, Graham and Gasser, 1931). The independence of the several effects observed is further emphasized if the analysis includes also the consideration of the demarcation potential and of the conduction velocity (p. 737).

It is likely that the broad correlations suggested by this and many other previous studies are not casual coincidences. The possibility of obtaining independent variations of the several properties, however, indicates that each property may be modified by other as yet unknown factors (cf. Graham and Gasser, 1931).

SUMMARY

The responses of circulated cat's peroneal nerves were recorded after intra-arterial injections of veratrine.

Veratrine has inconsistent effects on the demarcation potential (p. 737). It decreases the conduction velocity (p. 737). It causes repetitive discharges in response to brief single stimuli (figs. 1 to 5). It increases the negative afterpotential (figs. 6 to 8). It results in a decrease of spike potential magnitude (figs. 9 and 10). It elicits alternation of responses at

certain frequencies of stimulation (fig. 11). It first augments and later depresses the resting electrical excitability (p. 745). It alters the normal relationship between the thresholds for make and break stimulation by direct current pulses (p. 745). Large doses reversibly abolish all responses (p. 747).

A broad correlation was found among negative afterpotential, repetition, supernormality, and decrease of spike potential amplitude. This correlation, however, had many exceptions. Each of these features could vary independently (fig. 3; pp. 748–750).

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THE MEASUREMENT OF GLUCOSE *Tm* IN THE NORMAL DOG

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A cellular limitation in the renal tubular reabsorption of glucose is manifested by the inability to transfer more than a certain maximal quantity per unit time. For convenience we term this quantity *glucose Tm* and express it in milligrams per minute. In the normal, well hydrated dog, glucose reabsorption remains essentially complete with successive increments in plasma concentration until the *filtered load*[1] approximates *glucose Tm*. Further elevation of plasma glucose results in abrupt glycuresis and the quantitative excretion of the excess glucose. Under these circumstances glucose excretion is equal to the difference between the *filtered load* and *glucose Tm* (1). The usefulness of a *Tm* measurement in physiological investigations depends to a large extent upon its stability and reproducibility. This report presents an examination of the *glucose Tm* with specific reference to these qualities.

EXPERIMENTAL PROCEDURE. Seven healthy, well-trained female dogs were used. In general the experimental procedure was the same as previously described (1). The plasma level was quite constant during any series of observations except in those which specifically examined the effect of a changing plasma level. As a routine the absolute plasma concentration was sufficiently high that the *filtration load* was at least 1.5 times *glucose Tm*. The hydration of the animal was assured by the preliminary administration of 50 ml. of water per kilogram. This was an adequate fluid reserve in those experiments where the urine output exceeded the infusion rate. The experiments which simply evaluated *glucose Tm* followed the routine of the first three periods of the experiment shown in table 1.

Glomerular filtration rate was assumed to be equal to the plasma creatinine clearance. Tubular reabsorption of glucose was calculated as the difference between the *glucose load* and its concurrent rate of excretion. A correction was made for renal dead space if there was a rapid change in plasma concentration. This was measured in our animals by determining

[1] In milligrams per minute this is equal to the product of the rate of glomerular filtration (ml. per min.) and the plasma glucose concentration (mg. per ml.).

the amount of clear urine excreted following the intravenous injection of 10 ml. of concentrated cyanol solution. The dead space was roughly proportional to urine flow over a considerable range and was quite close to the amount of urine formed in two minutes (mean = 2.23 min.).

EXPERIMENTAL RESULTS. *The influence of changing plasma glucose concentration on its renal tubular reabsorption.* In a series of 13 experiments *glucose Tm* was measured in three periods at a constant elevated plasma glucose concentration; the glucose in the infusion fluid was then removed or decreased in concentration and additional observations made as the

TABLE 1

An experiment which examines the effect of falling plasma glucose concentration on the renal tubular reabsorption of glucose in the normal dog

6/14/37; dog G:

0 time	1000 ml. water by stomach tube
30–40	3.0 grams creatinine, 25 grams glucose in 150 ml. of 0.85 per cent NaCl solution
40–116	Constant infusion started at the rate of 6.0 ml. per minute. Glucose 20 per cent, creatinine 0.6 per cent made up in 0.85 per cent NaCl
85	Initial period started
116–end	Infusion continued at rate of 6.0 ml. per minute. Creatinine 0.6 per cent made up in 0.85 per cent NaCl

PERIOD NUMBER	CONCURRENT TIME	URINE FLOW	PLASMA LEVEL		CREATININE CLEARANCE	GLUCOSE		
			Creatinine	Glucose		Filtered	Excreted	Reabsorbed
	min.	*ml. per min.*	*mgm. per cent*	*mgm. per cent*	*ml. per min.*	*mgm. per min.*	*mgm. per min.*	*mgm. per min.*
1	85–94	18.6	35.2	666	80.1	533	313	220
2	–104.5	14.4	33.8	648	77.6	503	293	210
3	–113	10.2	33.2	624	84.5	527	302	225
4	120–128.5	4.2	31.9	467	82.2	384	172	212
5	–137.5	2.1	31.6	332	82.8	275	41	234
6	–148	1.6	31.6	220	83.2	183	8	175
7	–165	1.3	31.6	175	85.9	150	0*	150

* Less than 0.5 mgm. per minute.

plasma concentration was falling (table 1, fig. 1). *Glucose Tm* was taken as the mean of the three initial observations. Reabsorption in the subsequent periods was calculated as the ratio of the observed rate to *glucose Tm* (i.e., T/Tm). In figure 1 these ratios are plotted against the *filtration load* for the period, also expressed in terms of *Tm* (i. e., *load*/*Tm*). In other experiments, observations on a rising plasma glucose were contrasted to subsequent periods when the plasma concentration was falling. In still others, observations on a rising curve were compared to subsequent periods at the high plasma concentration.

The experiments of the first group (fig. 1) are the only ones which suggest that glucose reabsorption may be conditioned by the direction and rate of change in the plasma glucose[2]. Even in this group the effect is neither constant nor extensive. The ratio T/Tm on a falling plasma glucose is significantly below 1.0 in a few experiments but the mean of the entire group is 0.941 ($\sigma = \pm 0.070$, 37 observations) and there is extensive overlapping between these and the control observations (1.00 ± 0.53, 36 observations). This is an uncertain demonstration that a rapidly falling blood glucose level influences reabsorption. It is our belief that the mechanical factor of renal dead space is quantitatively more important

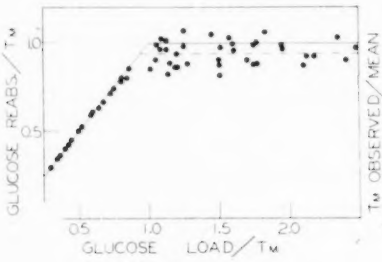

Fig. 1

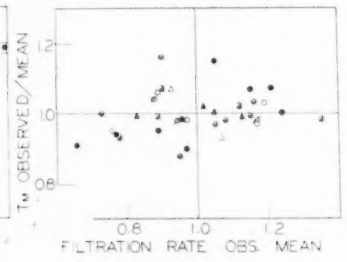

Fig. 2

Fig. 1. The effect of rapidly falling plasma concentrations of glucose on its renal tubular reabsorption.

The control periods which serve as the standard of reference in each experiment are not shown. Each dot represents the ratio observed in a single experimental period. Periods in which the ratio load/Tm spans the value of 1.0 have been omitted.

Fig. 2. Showing the lack of correlation between glomerular filtration rate and *glucose Tm*.

Each point represents an experiment of three or more periods. The mean glomerular filtration rate and *glucose Tm* has been calculated for each animal. The values for each experiment have been plotted as the fractions of these means. The symbols used to represent each of the animals are as follows: A, dots; H, circles; B, open triangles; G, filled triangles; CT, half filled squares, C, half filled circles; P, crossed circles.

than this. The latter makes it difficult to obtain a plasma concentration which is representative of the period. However, the necessity for a constant plasma glucose in the measurement of *Tm* is derived from both factors.

The influence of excess insulin on glucose reabsorption. This was observed in six experiments. In each of these, a series of three control periods was obtained at an elevated plasma glucose level, 50 units of insulin were administered intravenously, and after an interval varying from 0 to 50 minutes a second series of observations was made. A summary of these

[2] The rate of change of plasma glucose in most of these experiments was comparable to that shown in table 1.

experiments is given in table 2. A depression of 10 per cent or more in the reabsorption of glucose was demonstrated in 4 of 6 experiments.

The influence of adrenaline on glucose reabsorption. We have not examined this in the dog, since there is an adequate series of observations on man (2). These experiments were similar in design to the insulin experiments. There was no change in *glucose Tm* which could be related to the influence of adrenaline on the transport system.

The reproducibility of glucose Tm. The measurements of *glucose Tm* in a series of normal dogs[3] subjected to repeated examination are given in table 3. These are sufficiently numerous in 5 dogs to define the variability which may be expected over a period of months. No precautions were taken to control the diet, nutritional status or general activity of the

TABLE 2

The influence of a large intravenous dose of insulin on the renal tubular reabsorption of glucose in the normal dog

DOG NUMBER	BEFORE INSULIN				AFTER 50 UNITS INSULIN INTRAVENOUSLY					RATIO, GLUCOSE REABSORPTION AFTER INSULIN / BEFORE INSULIN
	Number of periods	Creatinine clearance	Plasma glucose	Glucose Tm	Number of periods	Time after insulin	Creatinine clearance	Plasma glucose	Glucose T	
		ml. per min.	*mgm. per cent*	*mgm. per min.*		*min.*	*ml. per min.*	*mgm. per cent*	*mgm. per min.*	
A	3	77.0	444	206	3	48-97	86.3	261	181	0.88
	3	48.4	857	194	4	29-87	52.0	764	173	0.89
H	3	62.4	878	252	3	44-96	64.0	915	220	0.87
	3	83.1	467	243	4	32-90	84.6	545	257	1.06
	3	53.1	999	227	5	16-95	52.9	1213	174	0.77
B	3	67.8	827	220	6	0-93	69.4	803	212	0.96

animals except in the case of dog C. The protein content of the diet was varied considerably in this animal with no effect upon *glucose Tm* (general mixed diet 5/1/37 to 5/19/37; high protein diet 5/19/37 to 5/28/37; low protein diet 5/29/37 to 6/9/37; general mixed diet 6/10/37 to 10/26/37, see *3* for composition of diets). We did not observe the changes in glomerular filtration rate which usually result from such dietary changes (3, 4, 5). However the maintenance of a high filtration rate may be attributed to the generous use of intravenous fluids.

[3] Dog P died two days after the last experimental observation. At the time of this experiment (6/11/37) he gave no evidence of distress. The lowering of *glucose Tm* in this experiment may be a true expression of variability in the normal animal; however, we believe that this experiment should be withheld from inclusion in the general consideration of the data.

TABLE 3

Demonstrating the reproducibility of glucose Tm in the normal dog when repeated examination is made over a period of months

DOG	DATE	NUMBER OF PERIODS	GLOMER. FILTRATION RATE	GLUCOSE LOAD / Tm	GLUCOSE Tm	GLUCOSE Tm OBSER. / Tm MEAN	GLOMER. FILTRATE PER MGM. Tm
			ml./min.		*mgm./min.*		*ml./gm.*
A	6/ 7/39	3	77	1.66	206	1.00	0.374
	6/12/39	3	48	2.12	194	0.94	0.247
	7/10/39	3	41	2.55	190	0.92	0.216
	7/16/39	4	55	3.25	195	0.95	0.282
	7/24/39	3	75	2.03	220	1.07	0.341
	7/31/39	3	60	2.21	185	0.90	0.324
	6/14/40	3	65	1.70	236	1.15	0.275
	6/24/40	3	71	1.82	220	1.07	0.323
			62		206		
H	6/ 5/39	3	62	2.16	252	1.06	0.246
	6/ 9/39	3	83	1.59	243	1.03	0.342
	6/26/39	3	53	2.31	227	0.96	0.233
	7/19/39	3	68	2.61	233	0.98	0.292
	6/18/40	3	82	1.29	231	0.97	0.355
			70		237		
B	6/21/39	3	68	2.55	220	1.07	0.309
	7/12/39	3	78	2.47	191	0.93	0.408
			73		205		
CT	6/20/40	3	76	1.83	276	0.93	0.275
	6/28/40	3	87	1.99	293	0.99	0.297
	12/26/40	3	88	1.53	317	1.07	0.277
	1/24/41	3	109	1.35	301	1.02	0.362
	1/30/41	3	132	1.72	291	0.98	0.454
			98		296		
G	5/14/37*	6	78	†	220	1.03	0.356
	5/26/37	5	87	†	208	0.98	0.418
	6/14/37	3	81	2.39	215	1.01	0.376
	10/ 4/37	3	64	2.19	213	1.00	0.300
	10/20/37	3	74	2.73	211	0.99	0.349
			77		213		
C	5/19/37	6	128	†	285	1.03	0.449
	5/21/37	4	99	2.23	321	1.16	0.308
	5/28/37	3	126	2.96	274	0.99	0.460
	6/ 7/37	5	104	†	245	0.88	0.425
	6/ 9/37	7	115	†	268	0.97	0.429
	10/11/37	2	80	2.25	276	1.00	0.290
	10/25/37	3	119	2.18	272	0.98	0.438
			110		277		

TABLE 3—*Concluded*

DOG	DATE	NUMBER OF PERIODS	GLOMER. FILTRATION RATE	GLUCOSE LOAD / Tm	GLUCOSE Tm	GLUCOSE Tm OBSER. / Tm MEAN	GLOMER. FILTRATE PER MGM. Tm
			ml./min.		*mgm./min.*		*ml./gm.*
P	5/10/37	3	97	1.44	332	0.98	0.292
	5/24/37	4	89	2.72	355	1.04	0.251
	5/31/37	3	118	2.03	333	0.98	0.355
	6/11/37	3	86	3.09	263*		0.327
			101		340		

* Died 2 days later.[3]

† Observed at a series of values for Load/Tm.

The reproducibility of *glucose Tm* in any one animal is quite striking. The variation is more than ±10 per cent of the mean for any animal in only 3 of 35 observations. This consistency is quite impressive since the measurement itself has an error in the order of magnitude of ±5.0 per cent. The mean *glucose Tm* derived from any three consecutive experiments would be quite adequate as a standard of reference for the study of subsequent change and a difference of 10 per cent or more would have significance if it were capable of consistent reproduction. The independence of glomerular filtration rate and *glucose Tm* previously noted in a limited series of observations (1) is clearly evident in the present data (fig. 2).

Discussion. Relatively few precautions need be observed in measuring *glucose Tm* in the dog because of the stability which characterizes the system. The insulin experiments suggest that an extensive change in cellular metabolism may be a factor in conditioning the transport mechanism. However, the absence of any change when adrenaline is administered or when the dietary regime is extensively varied minimizes the practical significance of this. The constancy and the absolute level of plasma glucose are obviously important. Rapid changes in plasma concentration should be avoided if only to prevent an error due to renal dead space. An additional reason lies in the possibility that such changes may in themselves affect the activities of the system. Any absolute concentration which results in frank glycuresis will be adequate to saturate all the nephrons[4] in the normal, well hydrated dog since further elevation does not result in any increase in glucose reabsorption (1). When the measurement is applied to abnormal material higher plasma concentrations are advisable since this relationship between glomerular and tubular function may be altered (2).

[4] This is an interesting finding since there is marked variability in the length of the proximal tubules in the dog (10). One may infer from this that there must be proportionate variation in glomerular development. If this were not so the precise balancing of tubular and glomerular function in the individual nephrons which contribute to total renal function could not occur.

The reproducibility of the *glucose Tm* is sufficient that it may be accepted as an excellent method for the characterization of the kidney. It is a quantitative functional measurement[5] of the tubular tissue available for glucose reabsorption (presumably proximal) under the conditions of the experimental rountine; hence the tissue with operating glomeruli (6, 7). It is to be stressed however, that the expansion of plasma volume which accompanies the maintenance of an elevated plasma glucose may bring into action glomeruli which otherwise would remain closed.

The relatively constant *glucose Tm*, despite variations in glomerular filtration rate, is in keeping with the histological evidence that essentially all the nephrons are continuously active in the normal dog (8). The absence of an increase in glucose reabsorption with progressive elevation of plasma glucose is also in favor of this interpretation. It seems likely that if there were a considerable number of inactive nephrons at moderate plasma levels some of these would be serviced by blood when the circulatory system is expanded by the high infusion rates necessary to produce severe hyperglycemia (1). In this view, variations in glomerular filtration rate such as we have observed are due to changes in the filtration pressure of the entire glomerular bed rather than to changes in the number of active nephrons. It may be possible that under certain circumstances nephrons can be withdrawn from activity as seems to be true in the avian kidney (9) and that some of the larger variations in our data may be the result of this. However, large variations in *glucose Tm* were too infrequent in our data to make this a safe conclusion at the present time.

SUMMARY AND CONCLUSIONS

1. The glucose reabsorptive system, as evaluated by *glucose Tm*, has considerable stability in the normal dog. Although we have observed

[5] Our data are too meagre to establish a correlation between *glucose Tm* and body weight, surface area, or kidney weight. The variation in *Tm* per gram of renal tissue is quite surprising. This warrants specific examination over a wider range of both variables and should include other methods of expression of tissue mass. The data pertinent to this comparison which are at hand follows:

DOG	GLUCOSE Tm	BODY WEIGHT*	SURFACE AREA*	KIDNEY WEIGHT
	mgm. per min.	*kgm.*	*sq.m.*	*grams*
A	206	14.0	0.60	100
H	237	17.0	0.69	138
B	205	16.5	0.68	104
CT	296	23.0	0.92	152
G	215	17.0	0.78	148
C	277	24.0	1.03	
P	340	26.0	1.04	

* When first examined.

some depression when the plasma glucose falls precipitously, this is slight and inconstant. Adrenaline has no effect upon glucose transport and one must use excessive doses of insulin to produce a significant depression and then it is not a constant phenomenon.

2. For the valid measurement of *glucose Tm* it is essential to work at a constant plasma level of adequate absolute concentration and to provide for the adequate hydration of the animal. It seems unlikely that close control must be maintained over other variables.

3. The reproducibility of *glucose Tm* measured under these conditions is excellent. It is recommended as a dependable means for the quantitative characterization of tubular (proximal) reabsorptive function.

4. Contrary to the constancy of *glucose Tm* the rate of glomerular filtration varied widely in these experiments. This feature of the data and other considerations indicate that under the conditions of our experiments essentially all the glomeruli are functioning and variations in filtration rate are the result of changes in the filtration pressure of all glomeruli rather than in the number of active nephrons.

APPENDIX. *Chemical methods.* The handling of the samples and the precautions taken in the analyses were the same as in our previous report (1). The chemical methods used in the experiments on dogs C, G and P were also the same. For the remaining dogs these were as follows: Plasma filtrates were prepared in 1:10 dilution using the ferric sulfate-barium carbonate method of Steiner, Urban and West (11). Excess barium in the filtrate was removed by the addition of minimal quantities of sulfuric acid, CO_2 was then shaken off and the pH adjusted to 7.0 with phenol red and sodium hydroxide. The urines if not contaminated by protein were not precipitated. Creatinine was determined by a modification (4) of Folin's alkaline picrate method. The optical density of each sample was determined exactly 10 minutes after the addition of the alkaline picrate. True glucose was determined as the difference between the reducing power of the samples before and after absorption on yeast (12) using the method outlined below. All colorimetric determinations were made with the Evelyn photoelectric colorimeter.

The Folin (13) sugar method proved to be unsatisfactory for adaptation to the photoelectric colorimeter. The reagent deteriorates rapidly, as evidenced by a progressive change in the slope of the standardization curve and there is a fairly rapid change in optical density after the addition of the acid phosphomolybdate and subsequent dilution. Furthermore the line relating optical density to glucose concentration does not extrapolate to 100 per cent transmission at zero glucose concentration.

Our modifications circumvent these difficulties to a large extent and introduce flexibility with respect to glucose concentration. In its present form, however, the method is not recommended for general application. Its most serious fault is its sensitivity to a change in the carbonate: bicarbonate ratio, a characteristic of copper reagents of this general type. For this reason it is essential that plasma proteins are precipitated by a method which yields a filtrate essentially neutral and with no significant buffer capacity. The method described above satisfies these requirements.

Solutions. The alkaline copper solution has the following composition:

Reagent I

Sodium carbonate ($Na_2CO_3 \cdot H_2O$)	15.0
Sodium tartrate	16.0
Copper sulfate ($CuSO_4 \cdot 5H_2O$)	5.0 (50 ml. 10 per cent solution)
Sodium bicarbonate	10.0
Water to 1.0 liter.	

This is prepared as directed by Folin except that the copper sulfate is added at the time the tartrate reagent is made. Some reduced copper usually precipitates in the first few days, but this may be disregarded if care is exercised when removing the supernatant fluid. The reagent is quite stable despite slow changes in the boiled blanks. Standardization curves are reproducible for a period of 5 or 6 months, which is as long as we have observed any one sample of reagent.

The acid molybdate solution is made up as directed by Folin except that the strength of sulfuric acid is increased by 30 per cent. This tends to stabilize the reduced phosphomolybdate color, although the degree of stability varies to some extent with different lots of reagent. The drift in optical density is generally less than two per cent in the interval of 20 and 30 minutes after the addition of the dilute phosphomolybdate reagent. We have routinely read our determinations from 20 minutes, on wards.

The determinations are carried out as directed by Folin with the following exceptions. There is no preliminary adjustment of the pH of the test solution in the sugar tube prior to the addition of the copper reagent, the boiling time is taken as 10 minutes, and before reading the samples are permitted to stand for 20 minutes after mixing with the dilute acid phosphomolybdate. With each set of sugar determinations three boiled blanks (i.e., water and copper solution) are included and 100 per cent transmission taken from the tube most representative of the three. The variability encountered in the blanks enters as a serious difficulty only in the lower ranges of concentration (i.e., 2.5 mgm. per cent or lower).

Standardization curves are constructed as usual with aqueous solutions of glucose using the no. 635 filter for low concentrations (1.0–5.0 mgm. per cent) and the no. 540 filter for the higher ranges (2.5–15 mgm. per cent). The absorption curve of the reduced phosphomolybdate is such that no error results from the use of a wave length quite removed from the absorption maximum. Recoveries of glucose added in known amounts to plasma or plasma filtrates and submitted to our procedures were usually well within ± 2.0 per cent of the theoretical. Reproducibility in practice was also within these limits. When proper care is taken in calculating dilutions, all samples can be read with the no. 540 filter.

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